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HIBERNATION AND MARMOT PHYSIOLOGY

BY
FRANCIS G. BENEDICT

Director, Nutrition Laboratory, Carnegie Institution of Washington

AND
ROBERT C. LEE



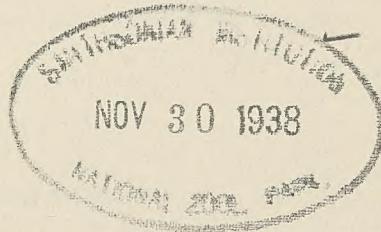
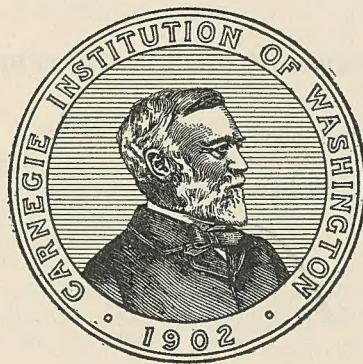
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INTRODUCTION

Hibernation is of chief interest and value in the comprehensive comparative survey of basal metabolism being made at the Nutrition Laboratory, for the reason that animals belonging to the hibernating class represent an intermediate step between the cold-blooded and the warm-blooded series. For comparisons of the vital activities of animals varying so widely in their physiological organizations as the cold-blooded and the warm-blooded animals, on the one hand, and animals in the hibernating and non-hibernating conditions, on the other hand, the best common measurement is that of the heat production. Consequently our investigation on hibernation centered around the main theme of vital energetics, with emphasis chiefly upon the heat production and to a less extent upon the heat loss.

Of the many hibernating animals, each with its own specific characteristic habits in hibernation, that animal most available in the northeastern section of the United States is the marmot (*Arctomys monax*) or woodchuck. This ranges in adult weight from 2 to 5 kg. and hence is comparable in size to many other species of warm-blooded animals that have already been studied in the normal condition. However, our main object in undertaking the marmot research was not to determine the basal metabolism of another 5-kg. animal. In this we were only incidentally interested. Our primary concern was to compare the heat production of this animal in the non-hibernating condition with its heat production in the hibernating state. Certain other comparisons were likewise made, specifically the comparison of the non-hibernating marmot with the ordinary warm-blooded animal and that of the hibernating marmot with cold-blooded animals when the cell temperatures of both were the same.

The literature on hibernation is more voluminous than accurate. In view of the importance of hibernation in studying vital activity, it is surprising that so few data of a useful, accurate nature are available. Most of the earlier literature deals with superficial observations regarding changes in body weight and respiration rate. The general appearance of animals in and out of hibernation and the measurements that have been made of the respiratory gases often have led to wholly erroneous conceptions as to the respiratory quotient and particularly its interpretation. The barrenness of the early literature has been admirably emphasized by Rasmussen.¹ Of the largest contributions, those most commonly cited are that of Dubois² and that of Polimanti.³ Some of the most accurate researches recorded in the literature were of invaluable aid in analyzing our observations on serpents, particularly

¹ Rasmussen, A. T., Am. Naturalist, 1916, 1, p. 609.

² Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896.

³ Polimanti, O., *Il Letargo*, Roma, 1912.

the reports of Mareš,⁴ Nagai,⁵ Hári,⁶ and Aszódi,⁷ which have been cited *in extenso* in our monograph on cold-blooded animals.⁸ Since the time of Rasmussen's article there have appeared many articles dealing with hibernation, a large proportion of them representing morphological studies and hence not normally to be included in the themes we studied. It has been deemed impracticable to cite in this report all the literature on hibernation, although the most pertinent literature will be referred to in our discussion of the various topics investigated.

In including the hibernating animal as an integral factor in our comprehensive, comparative survey of metabolism, we have maintained and continued a principle long followed by the Nutrition Laboratory, that is, to confine our researches to normal physiology and to introduce only a minimum of pathology, pharmacology, and operative procedures. For this reason the Nutrition Laboratory is not in a position to emphasize particularly studies on the anatomy and the histology of the marmot. We have designedly concentrated our efforts upon a study of the heat production and its associated factors of body temperature, respiration rate, heart rate, and vaporization of water. With the completion of the comprehensive study of cold-blooded animals, the results of which have been published by the Nutrition Laboratory from time to time, the transitional or intermediate phase of animal life between the cold-blooded and the warm-blooded animals assumed an unusual interest. It is possible, by means of curare or certain operative procedures, to transform a warm-blooded animal into a state approximating that of the cold-blooded animal. The findings of such types of study, however, have been anything but conclusive and convincing. It is far simpler, on the other hand, to raise the cell temperature of the cold-blooded animals up to the temperature of the warm-blooded animals by warming them. The findings obtained in this type of study are important but limited, for very few cold-blooded animals, with the exception of the snake, can endure having their body temperature increased to that of the warm-blooded animal, *i.e.*, 37°C., and for only a relatively short time. These procedures are usually of a transitory nature. An animal can be warmed or cooled, but usually it cannot withstand such changes in body temperature for any length of time, and rarely can the experiment be repeated on one and the same animal, as frequently the animal dies as a result of such treatment. Hibernation, on the contrary, is a normal, natural physiological process. It is reproducible. The animals can pass from one extreme phase to another, back and forth, almost at will, can be studied at both extremes, that is, when hibernating and when awake, and also, although not so accurately, can be studied in the transitional phases. Hence a most important gap in

⁴ Mareš, F., Bohem. Arch. Med., Journ. Advance. Med. Sci., Prague, 1889, **2**, p. 458 (English translation on file in the Nutrition Laboratory); *idem*, Compt. Rend. Soc. de Biol., 1892, 9th ser., **4**, p. 313.

⁵ Nagai, H., Zeitschr. f. allg. Physiol., 1909, **9**, p. 243.

⁶ Hári, P., Biochem. Zeitschr., 1921, **113**, p. 89; *idem*, Arch. f. d. ges. Physiol., 1909, **130**, pp. 90 and 112.

⁷ Aszódi, Z., Biochem. Zeitschr., 1921, **113**, p. 70.

⁸ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 425, 1932.

our knowledge of the comparative metabolism of animal species can be filled in by observations on this type of animal.

At one time in its yearly life the marmot "approximates" the warm-blooded animal and at another time the cold-blooded animal. In the extensive report from this Laboratory in which snakes and tortoises were compared with warm-blooded animals, computations based on the earlier work on the hibernating marmot, chiefly that of Mareš and that of Nagai, showed that when this warm-blooded animal was cooled in hibernation to the temperature of the cold-blooded animals its metabolism was very low, but actually was never so low as that of the cold-blooded animal of the same size. Under such conditions we may state that the hibernating animal "approximates" the cold-blooded animal, but in view of its intense normal activity, the fact that it has definitely the appearance of a warm-blooded animal and a rectal temperature essentially that of a warm-blooded animal, it is perhaps more proper to state that the marmot "approximates" the warm-blooded animal. As the treatment in our report on snakes shows, the metabolism of the marmot is higher per unit of weight or per unit of surface area than that of the cold-blooded animal with the same cell temperature. Hence the statement that the hibernating marmot is one day a warm-blooded animal and the next day or a week later is a cold-blooded animal is only partially true. The disposition of the marmot to approximate these two general classes, indeed to oscillate between them, suggested the possibility that this animal might represent figuratively the missing link or the bridge spanning the wide gap existing in physiological activity between the cold-blooded and warm-blooded series of animals. The early literature contains many observations contributing data on this point. They are, however, far from being complete. As the Nutrition Laboratory has made an extensive study with cold-blooded animals and with warm-blooded animals, the latter ranging in weight from 8 grams (dwarf mouse⁹) to 3672 kilograms (elephant¹⁰), we felt it highly desirable to determine whether there is any animal whose metabolism would bridge the gap between the metabolism of the warm-blooded and that of the cold-blooded animal. The attempt to establish such a bridge had frequently been made before. Our effort to study the very few cold-blooded animals that can withstand a cell temperature as high as that of man showed that the rattlesnake and the python, when warmed to a temperature of 36° or 37° C., had a metabolism that did not approximate to any degree that of the warm-blooded animals. Hence from the standpoint of comparisons at the same cell temperature this type of bridge was wholly unsuccessful, and the conclusion was reached that cell temperature as such is not the determining factor in metabolism.

Another method of attack in studying this problem is to lower the temperature of the ordinary warm-blooded animal to that of the cold-blooded animal and measure its metabolism under such conditions. This has been

⁹ Benedict, F. G., and R. C. Lee, *Annal. de Physiol.*, 1936, **12**, p. 983.

¹⁰ Benedict, F. G., *Carnegie Inst. Wash. Pub.* No. 474, 1936.

done by means of curare, but this procedure impairs the respiratory functions, involves artificial respiration, and the whole method of study is quite contrary to the fundamental principle of the Nutrition Laboratory of investigating problems uncomplicated by operative procedures. Such experiments have been carried out by Krogh,¹¹ Velten,¹² and Krarup,¹³ who made use of animals that had been narcotized either by curare or occasionally by urethane. When such warm-blooded animals are narcotized and the cell temperature is very much lowered, the animals usually die, as rectal temperatures below 24° or 25° are almost invariably fatal. This method of attack is, therefore, distinctly out of our field of work.

In our long series of studies on various animals, not infrequently conditions were met that at least suggested a correlation between lowered cell temperature and lowered metabolism, but in our purely physiological studies efforts were usually made to avoid these conditions. For example, in the rectal temperature studies with mice and rats it was concluded that if the rectal temperature was unduly low the metabolism was not basal but was lower than basal, and consequently such metabolism measurements could not be compared with strictly basal metabolism measurements. Thus we made every effort to avoid those very conditions which, for this present study, would have been helpful in throwing light upon the possibility of a bridge between the two classes of animals. Frequently, however, metabolism studies did accompany these observations on lowered cell temperature. As early as 1926 in a research on the rat a number of measurements were made on rats in what is termed a "moribund condition."¹⁴ The rats did not die suddenly but were growing constantly weaker. These observations showed a very low metabolism.

Anything contributing to a lowered metabolism challenges attention in all basal metabolism work, for the argument is continually being used in studying animals and men that it is perfectly possible and, indeed, only too easy to secure measurements above basal, chiefly due to restlessness or activity, but that it is impossible to secure measurements below basal. No one factor has been more continually looked for in our studies than a subnormal basal metabolism, this being really a wholly inconsistent expression. If the metabolism is subnormal, it would not be basal. However, we were continually searching for conditions that might be imposed upon an individual animal, bird, or even human, whereby the metabolism might be depressed. Obviously with humans this could not be done without endangering health. One of our associates, Dr. Fritz B. Talbot, however, was able to study an infant having a congenital absence of the two brain hemispheres.¹⁵

¹¹ Krogh, A., *Internat. Zeitschr. f. physik.-chem. Biol.*, 1914, **1**, p. 491; *idem, The respiratory exchange of animals and man*, London, 1916, p. 94.

¹² Velten, W., *Arch. f. d. ges. Physiol.*, 1880, **21**, p. 361.

¹³ Krarup, J. C., *The influence of temperature on the respiratory metabolism or heat production*, Dissert., Copenhagen, 1902 (In Danish).

¹⁴ Benedict, F. G., and G. MacLeod, *Journ. Nutrition*, 1929, **1**, p. 389.

¹⁵ Talbot, F. B., *Archives of Pediatrics*, 1915, **32**, p. 452.

He found the metabolism of this infant to be extraordinarily low, although the body temperature was not significantly lowered. This strongly suggests that the metabolism may be controlled by the brain, in which case animals with relatively the largest brains should have relatively the higher metabolism. The chief interest in this case for us, however, was the fact that the child without the brain hemispheres had a definitely low metabolism approaching the metabolic activity of a cold-blooded animal, although the rectal temperature had not descended significantly. Thus we see that in the matter of a bridge between cold-blooded and warm-blooded animals, a cold-blooded animal such as the snake does not have a metabolism corresponding at all to that of a warm-blooded animal even when its cell temperature corresponds to that of the warm-blooded animal. In fact, under such conditions the snake has only about one-eighth of the metabolism of the warm-blooded animal. Furthermore, the cell temperature *per se* is not the controlling factor in metabolism.

In the cold-blooded animal series the tortoise occupies an unusually interesting position, in that it has a metabolism significantly higher than that of the snake, the alligator, and the lizard, but comparison of the metabolism of an animal having the unique structure of the tortoise with that of another animal of an entirely different structure presents an almost insuperable difficulty. A 5-kg. tortoise is made up of a rather significant proportion, nearly 30 per cent, of what might be termed wholly inactive metabolic material, *i.e.*, the shell. Consequently a clear, uncontaminated comparison of a 5-kg. tortoise and a 5-kg. snake cannot be made, weight for weight. If we make the comparison on the basis of the tortoise weight less shell, for example, comparing the metabolism of a tortoise having 5 kg. of flesh weight with that of a 5-kg. snake, we find that on this basis the tortoise has a much higher metabolism than does the snake. As a large part of the skeleton of the tortoise is an integral part of the shell, one should really deduct from the total weight of the snake a part of its skeletal weight. By every method of computation it is definitely shown that the metabolism of the tortoise per unit of weight or per unit of metabolically active tissue is considerably higher than that of the snake. A plausible explanation is suggested in the fact that the flesh of the tortoise is much more richly supplied with blood vessels than that of the snake. The flesh of the snake, for example, is pale and contains an extraordinarily small amount of blood. It is yet to be demonstrated whether the color of the tortoise's flesh is due to residual blood in the capillaries and small veins or is simply due to pigmentation. If it is due to the blood, then one can properly conclude that the flesh is much more highly vascular and much more highly supplied with blood and, therefore, must be more able to support a high metabolism than the pale, colorless, almost bloodless flesh of the snake. The fact that the muscles of the tortoise are found to approximate much more those of warm-blooded animals in activity and general physiology than do those of the frog confirms this be-

lief.¹⁶ The question is far too important, however, not to demand further investigation. For example, do dark-fleshed fish such as the trout and salmon have a higher metabolism? These fish live at a much lower temperature than other fish. They can be active at practically 0° C., when other fish would be rigid and might possibly die. This capacity for life at low temperatures and probably a higher metabolism should be closely associated with a larger blood supply, and obviously experiments with this in mind are most desirable.

These points with regard to the shell of the tortoise and the pigmentation or blood supply of the flesh are cited simply to show the complexities of the attempt at the establishment of a bridge between the cold-blooded animals as a group and the warm-blooded animals as a group by warming the cold-blooded animal to the cell temperature of the warm-blooded. When the warm-blooded animal is cooled and the rectal temperature thereby significantly lowered as a result of the use of curare or an operative procedure, there is a trend in the direction of a lowered metabolism along with the lowered cell temperature, but the lowered rectal temperature is not accompanied by a proportionately great lowering in the metabolism. Hence these two methods of trying to construct a bridge between the cold- and warm-blooded animals might be compared to the building of a cantilever bridge across a river, both sides extending out but the two sides never hitting, the side from the cold-blooded animals being too low and the side from the warm-blooded animals being too high. The ruling out of these methods of comparison emphasizes even more the possibility of a hibernating animal representing this bridge, as its metabolism normally oscillates between that of the warm-blooded and that of the cold-blooded. A survey of the metabolism of the marmot with modern techniques and particularly with techniques perfectly comparable to those used for studying the metabolism of both warm-blooded and cold-blooded animals should be illuminating and might lead to a more successful establishment of a bridge. Although the indications of the earlier researches were distinctly to the effect that a perfect bridge could not be expected, nevertheless a complete survey of this animal under its various conditions is imperatively needed before any deductions can be drawn that such a bridge does not exist. If such a survey makes it clear that a bridge does not exist, will the observations on the metabolism of the marmot aid in explaining in any way the tremendous differences in metabolic activity of the cold-blooded and warm-blooded animals?

From the foregoing consideration it can be seen that a metabolic survey of the marmot should be a comprehensive one, for we have always a dual purpose in making observations on this animal, first, to contribute information regarding the physiology of the marmot itself, and secondly, to make use of such data in comparative physiology. From the standpoint of the marmot the usual information needed for each individual animal is necessary; from the standpoint of vital energetics evidently the important observations

¹⁶ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 425, 1932, p. 515, footnote 1.

should deal with the heat production under normal non-hibernating conditions, this study being accompanied by an adequate survey of the body temperature, the heart rate, the respiration rate, and the vaporization of water. Direct calorimetry is, we believe, no longer essential. A study of the metabolism of the goose¹⁷ under conditions of normal feeding, prolonged fasting, and surfeit feeding led to the conviction that by means of indirect calorimetry results of precisely the same value as obtained with the extremely complicated direct calorimetry were secured. Consequently in this study no direct calorimetric measurements were made. In the study of the basal metabolism of the marmot the zone of thermic neutrality should be established, and one should note the deviations in metabolism outside this zone, with special emphasis upon the heat measurements at the environmental temperature of 16° C. for the special purpose of making a comparison with certain other animals studied at this temperature level. No one factor has been more commonly used as a general physiological measure in Nutrition Laboratory researches than the resistance of an animal to prolonged fasting. The marmot, because of its heavy fat deposits and its naturally-imposed long winter sojourn without food, is especially suited to a study of its resistance to fasting.

By far the most important object, one for which the whole research was primarily undertaken, was the study of the marmot during the hibernating cycle. This included observations during the transition of the animal from the non-hibernating state to hibernation, with special reference to the metabolic activity during hibernation, and a careful study of the almost explosive nature of its heat production when the marmot is awakening from hibernation. Finally several efforts were made to contribute further information regarding the causes inducing hibernation and the possible explanation thereof, as shown in experiments with carbon-dioxide-oxygen anesthesia, and particularly by the use of nembutal narcosis. Consequently in the following discussion the results of our investigation will be considered essentially in the order outlined above, emphasis being laid first upon the non-hibernating marmot in order to contribute as much information as possible regarding its normal physiology, which will be of invaluable aid in interpreting the effects of the superimposed factors of prolonged fasting, hibernation, and special narcosis.

In concluding this introductory sketch, mention should be made of the obligation we are under to our colleague, the late Mr. Edward L. Fox, who carried out the orientation phase and obtained the early data in this research. His work formed a background without which we would have been greatly handicapped.

¹⁷ Benedict, F. G., and R. C. Lee, Carnegie Inst. Wash. Pub. No. 489, 1937.

TECHNIQUES EMPLOYED

ANIMALS USED

Because of the wild and ferocious nature of the marmot, special methods of handling had to be devised, and since there is a tremendous variability in its metabolic level, it has been necessary to give particular consideration to the technique.

Of the 48 marmots used for our research, varying in weight from 1 to 5 kg. on arrival, most were obtained from the northeastern section of the United States. Indeed, all but three came from New England. The two largest animals came from the state of Illinois.

The sex of only a few of the animals was determined. Animals B, C, M, 2 and 22 were males and animals A, D, E, O, P, 1 and 9 were females.

In the shipping container with animal O, which was received on April 30, 1935, two new-born marmots were found, each about the size of a mouse, one partly eaten. The animal was very ferocious, and only after escape and recapture was it transferred to a cage. Shortly after marmot O was placed in her cage, another half-eaten young was found. This experience would indicate that a litter may contain at least three.

Housing. Our animals were kept in a room in the laboratory building, when a controlled environmental temperature in the vicinity of 28° C. was desired. To obtain cold environmental temperatures suitable for hibernation, the animals were kept during the winter months in a cement garage not attached to any other building. Some animals were kept in heavy galvanized wire-mesh cages with individual partitions, others in Abderhalden cages, making urine collections possible. In the fall of 1936 a group of 15 animals was kept in a large galvanized iron chamber, open at the top. There was no noticeable fighting among this group of animals, and all improved in weight and appearance.

For the basal metabolism experiments, the temperature of the room where the animals were kept previous to the experiment was controlled at a level of $28^\circ \pm 2^\circ$ or 3° C., by means of a thermostat. The temperature was maintained at essentially 10° C. when the animals were in hibernation or when it was desired to have the animals hibernate. Occasionally the temperature was lowered to below 10° , and sometimes it rose considerably above 10° due to weather conditions.

Feeding. The earlier groups of marmots, those designated by letters, were fed largely on cabbage and bread. The numbered animals had cabbage and bread in addition to other vegetables, but their main food was a corn meal mash, made from canned cream-style corn mixed with corn meal. Our six-years' experience with 48 marmots showed that bread, cabbage, carrots, apples and corn on the cob were preferred to squash, beets, cauliflower, and potatoes, but in the absence of other food these vegetables were eaten. The

above-mentioned corn mash was the most popular food. The animals were fed daily, unless fasting was being enforced for experimental purposes. Water was practically always available to our marmots, even during enforced fasting, with the exception of a few hours prior to experiments in which water-vapor measurements were made. The care and feeding of these animals were in the hands of C. W. Hatch.

Handling. The smaller animals were handled by means of heavy leather gloves, being held by the back of the neck, but more frequently it was necessary to secure them by the neck or by the neck and one front leg with a noose formed by a leather thong fastened to the end of a sturdy pole. With gloved hands one could then safely grasp the animal's back. An attempt was made to have the animals wear collars and to snap chains to a ring on the collar, but this was found to be impracticable. The animal frequently snarled or caught the chain in the wire mesh of the cage, and if the chain was removed, it was impossible to fasten it on again without first securing the animal by some other means.

Animals in the hibernating state were handled with gloved hands, the gloves being used to prevent the transmission of the warmth from the hands to the animal. For many of the hibernating experiments, the animals were placed in the chamber and left for hours and in some instances a day or two before beginning the measurements, in order that the effect of handling would not complicate the results. Occasionally animals were found that could be handled fairly freely without awakening them from hibernation, but, in general, handling the hibernating marmot tended to waken it.

WEIGHING OF MARMOTS

The relatively simple operation of weighing would seem to leave no point for discussion in physiological measurements, and yet the weighing of the hibernating marmot has played such a great rôle in the historical development of its physiology that a critical analysis of this procedure is necessary. Presumably it has been repeatedly demonstrated that the marmot actually gains in weight during hibernation, when taking no food or water. This gain in weight has not been found invariably but nevertheless has been found so frequently that it is commonly stated to be a feature of the physiology of this animal. It, therefore, was necessary for us to perfect our technique in such detail as to insure that if an increase in weight was found, it could not be ascribed to any extraneous cause. The heavier animals were weighed on a platform balance with a sensitivity of approximately 10 gm. This is sufficiently accurate for animals weighing several kilograms and frequently either rapidly gaining or losing weight. Some animals were weighed upon a spring balance, in which case they were weighed with an accuracy of ± 7 gm. The spring balance was obviously much more rapid, and it was preferred when a large number of marmots were to be weighed.

The most accurate weighing was required when the animals were hibernating. To determine changes in weight, the animal was placed upon a Sauter

balance with a potential accuracy of one centigram. We felt convinced that if a gain in weight occurred, it could be noted with this balance. The relative humidity and temperature of the surrounding air play a great rôle with a heavily furred animal having a large hygroscopic area exposed to the air. But quite aside from the water held either by the hair or possibly retained at the base of the fur, we have the question of whether or not the container, cage, or respiration chamber in which the animal is weighed is maintained under conditions such that no possible changes in weight can take place. In the Haldane method of determining the respiratory quotient the changes in weight of the chamber plus the animal play a great rôle. The chamber is frequently large, and it is questionable whether or not changes in weight of the animal plus the chamber can be ascribed to changes in weight of the animal itself. A fairly large train of absorbing vessels containing soda-lime or sulphuric acid must be weighed in this type of Haldane gaseous metabolism measurement. Whether the changes in weight of these containers accurately measure the carbon dioxide absorbed or are in part due to actual changes in the weight of the container itself is to be sharply criticized.

If our apparatus could be weighed after an indefinitely long sojourn in an absolutely dry atmosphere and at a constant temperature, this question would play no significant rôle. The Nutrition Laboratory has had at least two striking experiences in which glass or metal containers had greater variations in weight than the so-called increase in weight of the marmot. It is a well-known fact that if an ordinary 125-mm. glass U-tube is cleaned and allowed to stand in the air for several hours before it is weighed, it is possible by means of a clean cheesecloth to wipe off as much as 50 mg. of condensed moisture, and this under conditions where no static electrical currents are discerned.

In the control of our emission calorimeter,¹ we attempted to secure absolutely anhydrous conditions inside the chamber. The chamber had nothing but metal inside of it, but by passing dry air through it moisture could be absorbed from the out-coming air current for hours, moisture not measured in terms of milligrams or centigrams, but in decigrams and not infrequently in grams. To be sure, the area of this chamber was large (entire volume, 125 liters), but nevertheless the amount of condensed moisture that may remain upon a metallic or glass wall of a chamber of this kind is such that it throws serious doubt upon a gain in weight being due to a gain in weight of the animal body.

In many of the earlier researches, however, it has been recorded that the animals were weighed, having been previously in a very dry atmosphere. In many cases the animals were taken from this dry atmosphere and weighed in the room, but the avidity of so-called non-hygroscopic material for water has only too frequently been entirely overlooked and an immediate gain in weight of the metallic or glass chamber is inevitably to be expected.

¹ Benedict, F. G., and R. C. Lee, Carnegie Inst. Wash. Pub. No. 489, 1937, pp. 14 and 215.

The procedure established by Pembrey of weighing against dummies can only be commented upon as a step in the right direction. That this, however, solves the problem is anything but certain. To establish the carbon-dioxide production and the oxygen consumption of an animal by using the differential of the weights of a train of absorbers, and of the respiration chamber together with an animal, as in the method of Haldane, where at best these quantities are in the order of milligrams, is, we believe, technically wrong and accounts for the greater part of those observations presumably demonstrating a gain in weight of the animal or an abnormally low respiratory quotient.

Weighings of the marmot are of importance from several standpoints, (1) to establish the normal course of the weight of the animal, either during partial or complete fasting and in a non-hibernating condition, (2) to determine the changes in weight during hibernation, and (3) to note the gains in weight following recovery or refeeding. Entirely aside from these series of weights, however, the body weights have further significance notably in connection with the so-called insensible loss. An animal that is resting quietly upon a balance without taking food or drink and without passing either feces or urine is continually undergoing a loss in weight, as has been so clearly demonstrated with humans. This loss in weight and the insensible perspiration have two rather important bearings, one in giving an approximate indication of water loss (because the chief loss is water) and the other in that with humans and some animals at least there is a close correlation between the insensible perspiration quantitatively measured and the metabolism determined simultaneously. This is emphasized at this point simply to stress that the weighing of the marmot, which is ordinarily simple, is more than ordinarily significant.

BODY TEMPERATURE

Measurement of the body temperature of a non-cooperative animal such as the marmot, which is aggressive when non-hibernating, presents the same difficulty noted in the measurement of the temperature of the rat or the mouse, that is, the difficulty of avoiding excessive struggle. This struggle consists of practically two distinct phases. The first is the inevitable struggle prior to or during capture. The second is the struggle after the animal is caught, while being held for insertion of the thermometer, and when the temperature is being obtained. To avoid struggle is practically impossible. Every effort was made to keep it at a minimum. Some animals became sufficiently accustomed to temperature measurements so that the struggle might be said to be minimized.

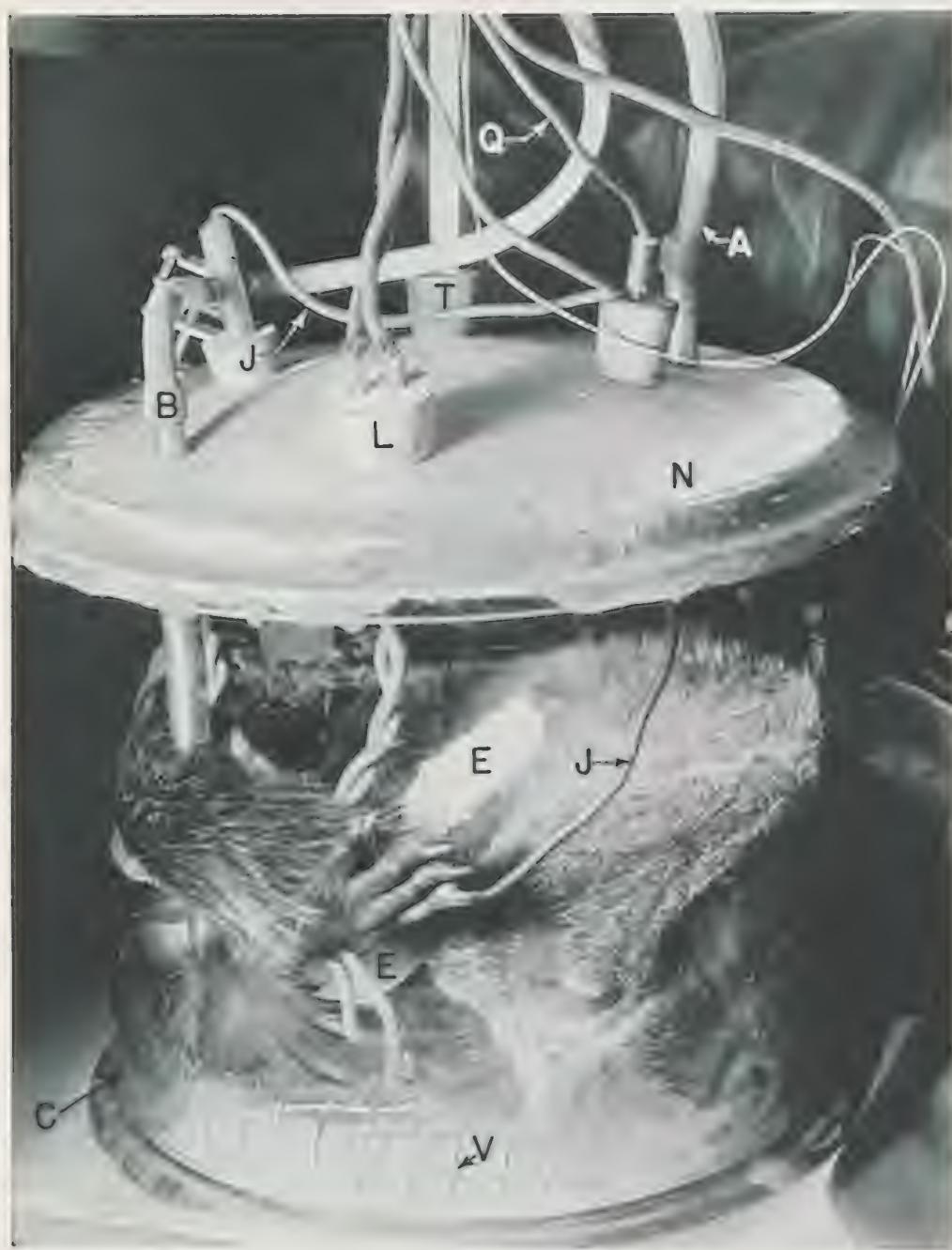
Either mercurial thermometers or electric thermo-junctions were used for body temperature determinations. These were calibrated and the correction factors applied. As there may be a temperature gradient in the rectum of an animal, both in the hibernating and the non-hibernating state, the depth to which the thermometer is inserted plays a considerable rôle. With

man this is commonly assumed to be 5 cm. With the non-hibernating marmot we invariably inserted the thermometer to a depth of 140 mm. or over twice 5 cm., by which we certainly measured as nearly as possible what one might term a central temperature uncomplicated by peripheral temperature.

In the taking of the temperature precautions were observed, based on other experiments. Many animals not hibernating were kept in a cold room to induce hibernation. It was found, in the study of humans, that if a thermometer no colder, for example, than a room temperature of 25° was inserted in the rectum or vagina, the mass of mercury and glass cooled the tissues locally. With humans we overcame this difficulty by placing the thermometer a small distance in the orifice, allowing it to obtain a constant temperature, and then inserting it several centimeters farther, under which conditions the true temperature was obtained. Too strong an emphasis cannot be laid upon this point, for in a recent study of the temperature of the feces of the elephant² the same factor entered. When marmots are in a cold environment at 10° or below, the difference between the temperature of the room and that of the non-hibernating marmot may be 26°. Under such conditions another method of temperature measurement was adopted. The thermometer was warmed in paraffin oil to essentially the temperature of the body and then quickly removed from the oil and placed in the rectum to the proper depth. The temperature was read as soon as the maximum was reached. The oil also served as a non-irritating lubricant.

These corrections and refinements of measurement apply only to the mercurial thermometer, which has a fairly large mass of mercury and glass. With the thermo-junction the situation was somewhat less critical, as the mass of the thermo-junction itself was relatively small and hence practically negligible. It was obviously impracticable to warm this thermo-junction previously in an oil bath, and this precaution was not taken. Instead, the thermo-junction was left in place long enough to insure the measurement of the true temperature. The objection to leaving the thermo-junction for any length of time in the rectum is that with non-hibernating animals this means sustained tension or possible struggle, which of itself would tend to raise the temperature. These particular precautions, however, deal primarily with the temperatures of the non-hibernating animals, when small differences in the body temperature may have some significance. Fortunately with the hibernating marmot practically all these refinements can be neglected, as the temperatures of the laboratory, the animal, and the thermometer will be nearly alike. The insertion of the thermometer results in no particular tension, although it does cause some stimulation, and the length of time for the thermometer to attain constancy plays no significant rôle, as the marmot is not struggling. The temperature measurements of the hibernating marmot do not have the complications occurring in the measurements of an active animal but are extremely accurate, which is

² Benedict, F. G., Carnegie Inst. Wash. Pub. No. 474, 1936, p. 138.

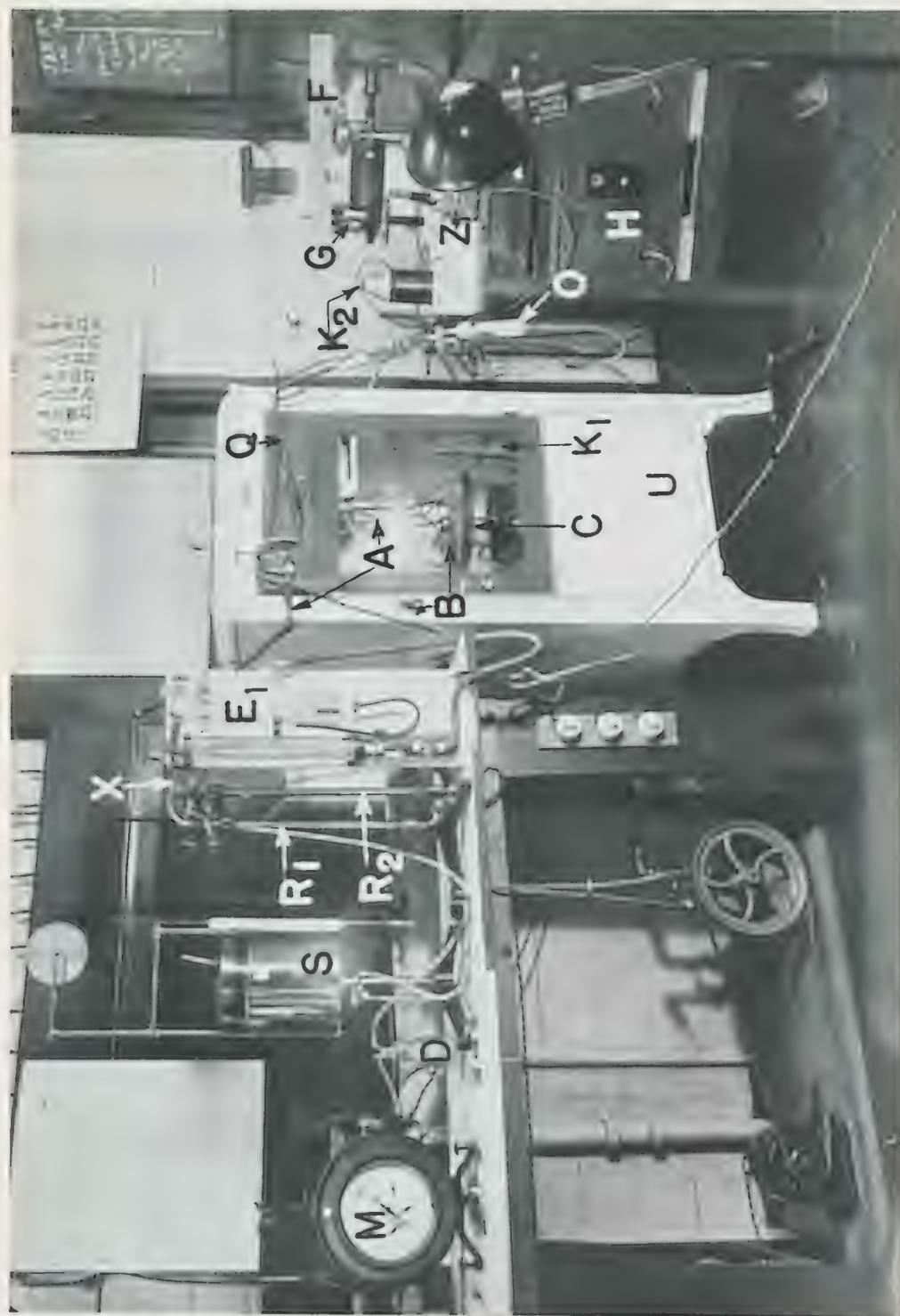


HIBERNATING MARMOT IN RESPIRATION CHAMBER

Hibernating marmot with tip of nose between E and E and with one eye directly under J, is shown curled up on wire grid, V, in chamber, C, with metallic cover, N; chamber thermometer, T; rectal thermo-junction lead, J; and heart rate leads, L, connecting with electrodes, E and E, on paws of animal. Air is introduced through ingoing-air-tube A and withdrawn through the outgoing-air-tube B, which extends to bottom of chamber. Special samples for respiratory quotients are withdrawn through capillary lead tube, Q.

APPARATUS FOR MEASURING THE METABOLISM, RECTAL TEMPERATURE, AND HEART RATE OF THE
HIBERNATING MARMOT

Dry air is supplied to chamber, C (seen through glass door of refrigerator, U), through ingoing-air-tube A. Air is withdrawn from the chamber by a small Collins blower (not shown in photograph) through tube B. Rotamesser R₁ or R₂ indicates the rate of flow of the ventilating air. Gas-analysis apparatus E₁ for controlling percentage of carbon dioxide in the chamber, connects directly with the outgoing-air-line by a glass tee at X. Aliquot samples of outcoming air (and in some cases the total outcoming air) are collected in 2.5-liter, oil-sealed spirometer S. Outgoing air passes through saturating bottle D before reaching wet gas meter M. Special samples for respiratory quotients are drawn through capillary lead tube Q to Haldane sampler O. The heart rate apparatus with amplifying unit H, Moll galvanometer G, with glass scale F, are seen at the right; also for rectal temperature determinations the reference thermometer and junction K₁ for cold temperatures and K₂ for warm or transition conditions, and portable galvanometer Z.





fortunate as they are of great significance in establishing whether the body temperature is correlated with the heat production. It is obvious that every degree change in body temperature, particularly at a low temperature level, may be of rather large moment. For example, if the Q_{10} law held, one would expect a change of possibly 25 to 30 per cent in the heat production per degree change in temperature, certainly at least 5 per cent per degree.

The electrical method (thermo-junction), which was used in most of the series of rectal temperature measurements with the hibernating marmots, has a great advantage over the mercury thermometer, in that the thermo-junction can be placed *in situ* and left there as long as the animal remains dormant, a procedure that enables numerous measurements.

In plate 1 the method of obtaining rectal temperatures during experiments on a hibernating marmot is illustrated by an actual photograph of an animal in deep torpor in a respiration chamber, where J is the thermo-junction lead by which the measurements are made. To obtain greatest accuracy in the electrical method at the hibernating levels, where the temperature was low, the reference junction and thermometer were also kept at approximately body temperature. This results in a small galvanometer deflection and, therefore, a smaller error in the measurement. The reference thermometer kept in the refrigerator is shown at K₁ in plate 2. The portable galvanometer used for these measurements is shown at Z.

RESPIRATION RATE

In physiological observations on most animals the respiration rate is usually studied either by noting movements of the chest wall or, with some animals, actually by the application of the respiration mask. With the marmot the application of the mask was out of the question, certainly with the non-hibernating animal. The movement of the chest wall is not so simple to observe because of the large mass of fur, and during the dormant or hibernating period the extremely slow respirations are difficult to count. Most of our measurements were made by observation of the movements of the chest or abdomen. In some experiments, with the marmots under nembutal, it was possible to adjust a partly inflated balloon in the body folds (see figure 1), so that each respiration of the animal would be registered outside the cage by a tambour and a kymograph. More significant than the respiration rate would have been the respiratory volume, but without a tracheal cannula or a mask this was out of the question.

HEART RATE

With the marmot a stethoscope could not be used to obtain measurements of the heart rate, due to the savage nature of the animal. This difficulty disappears when the animal is in hibernation, but in this condition the continuous or intermittent adjustment of a stethoscope would tend to waken the marmot. All the heart rate measurements were made with a modifi-

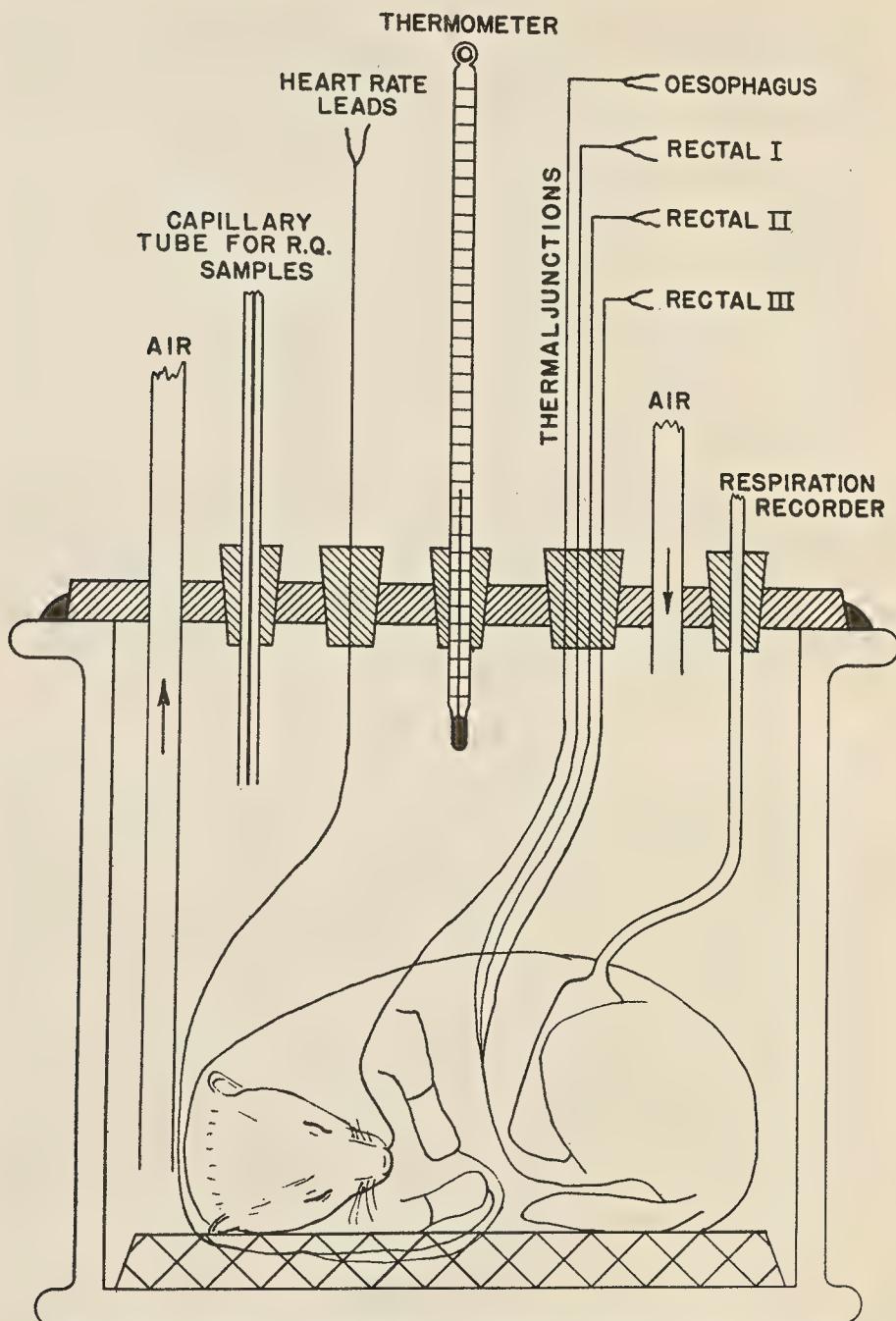


FIG. 1—DRAWING OF HIBERNATING MARMOT IN RESPIRATION CHAMBER

This drawing shows the glass respiration chamber with metallic cover, the thermometer, the ingoing- and outgoing-air-tubes, and the position of the various leads, which are introduced into the chamber by means of rubber stoppers. The animal is lying on a wire grid, which raises it from contact with the chamber. The electrodes are attached to the front paws, and the respiration recorder is in place.

cation of the Boas cardiotachometer³ and the use of electrodes, which were usually attached to the two front paws by means of adhesive tape or clamps. In plate 2 some details of the heart rate apparatus, as used for hibernating marmots, are visible. The details of the leads through the chamber cover and the electrodes strapped to the paws are shown in figure 1. With the exception of the first few early observations, a commercial electrode paste was used.⁴

In the determination of the heart rate of the non-hibernating marmot, one animal stood with each front foot on one of two wire gauze electrodes fastened to wooden paddles, but other animals required the wire gauze electrodes fastened to the paws by means of adhesive tape. The animals were kept in Abderhalden cages in a dark room. The apparatus and observer were in a room on a different floor, to prevent any noises that might stimulate the animal. Precautions were taken to continue measurements until no further decrease in heart rate occurred.

No attempts were made to photograph the character of the waves, and hence our records deal only with the actual heart rate. The disadvantage of the apparatus was that it required constant attendance of an observer. Without doubt photographic adjustments could have been applied, but in the transitional stage, waking from hibernation, the changes in rate were such that an observer making records intermittently could easily follow the course.

URINE COLLECTION

The animals from which urine was collected were all kept in Abderhalden cages, and a container with hydrochloric acid, and in some cases chloroform and thymol, was placed beneath for collection. The measured volumes included the washings and preservatives. All urines were acidified before they were measured and bottled. The nitrogen was determined by the method of Folin.⁵

BODY ANALYSIS

The body composition was determined with a few animals by first drying the flesh, which had been separated from the bones by boiling the carcass in water. By repeated vaporization of the water in a drying oven and weighing when cool until a constant weight was found, the dry weight of bones and flesh was obtained. The fat was determined by ether extraction of an aliquot portion, and nitrogen was determined by the well-known Kjeldahl method.

³ Boas, E. P., Arch. Intern. Med., 1928, 41, p. 403; Boas, E. P., and E. F. Goldschmidt, *The heart rate*, Baltimore, 1932. Modifications described by Benedict, F. G., Carnegie Inst. Wash. Pub. No. 474, 1936, p. 26.

⁴ Redux, sold by Sanborn Company, Cambridge, Massachusetts.

⁵ Folin, O., *Laboratory manual of biological chemistry*, New York, 5th ed., 1934.

METABOLISM MEASUREMENTS, NON-HIBERNATING

All the non-hibernating metabolism measurements were made by the open-circuit principle. This method has been well proven in the Nutrition Laboratory in earlier work. It entails ventilating a chamber of a size suitable for the particular marmot being used, with dry outdoor air, measuring the total ventilation by a calibrated meter of suitable capacity, and accurate collection of an aliquot sample which is subsequently analyzed on the extremely precise Carpenter gas-analysis apparatus.⁶ A schematic diagram of the apparatus is shown in figure 2. In addition to the determinations of the carbon dioxide produced and the oxygen consumed, measurement of the water vapor given off by the animal was frequently possible. Since the ventilation rate used

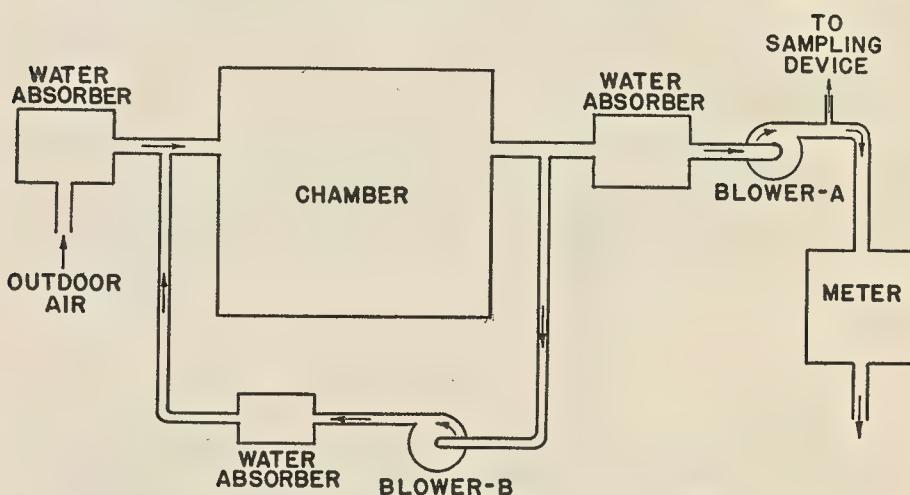


FIG. 2—SCHEMATIC DRAWING OF APPARATUS USED FOR GASEOUS METABOLISM MEASUREMENTS

Outdoor air, dried by water absorber, flows into chamber. Air, withdrawn from chamber by blower A, is dried by water absorber and measured by meter, the aliquot sample being obtained at point indicated. For water-vapor determinations supplementary ventilation is also used, blower B forcing air through water absorber and returning it to ingoing-air-line.

to obtain air having a carbon-dioxide content of approximately seven-tenths of one per cent (the optimum for the Carpenter gas-analysis apparatus) was not sufficient to carry off the water vapor fast enough to prevent condensation, it was necessary to employ an absorber with an auxiliary ventilating line or by-pass from the outcoming-air-tube back to the ingoing-air-tube to collect all the vaporized water. By weighing the water absorbers (usually containing calcium chloride) in the outgoing line and in the auxiliary line, the amount of water vapor produced in any given period could be determined. When water-vapor measurements were not attempted, the

⁶ Carpenter, T. M., Abderhalden's Handb. d. biolog. Arbeitsmethoden, 1933, Abt. IV, Teil 13, p. 593.

auxiliary ventilation line was pinched off near the main ventilating line. Dry outdoor air entered the top of the chamber and was removed from the bottom of the opposite side of the chamber by means of a small Collins blower. To permit consecutive determinations of the respiratory quotient, the sampling device consisted of two 2.5-liter spirometers, which could be used alternately. One of these is shown in figure 3. A small disk, D_1 , of suitable size, reduced the volume of the air permitted to pass through a branch in the ventilating air line, so that 1 liter or more of air was collected during a period, usually of 20 to 30 minutes' duration. The exact volume was determined by scale F_1 , and is used in computations. The ventilating air was measured usually by means of a wet meter, but in the case of some of the larger animals it was necessary to use a dry gas meter in order to measure accurately the ventilation at the higher rates. The sampling spirometers were filled with Squibbs mineral oil in order to prevent any change in the composition of the sample, even if stored for some time in the spirometer. In order to maintain a precisely constant ventilation rate two devices (not indicated in figure 3) were used in the flow line, a Murrill regulator,⁷ which has proved a most extraordinary device for maintaining a constant flow, especially for rates of flow of over one liter per minute, and a rotamesser which enabled the continuous observation of the rate of flow. The rotamesser was placed after the meter.

Frequent tests were made of the equipment to be sure that the apparatus was tight, and an alcohol lamp was placed in the chamber and analyses were made to insure the correct measurements of the changes in the composition of the air. As a satisfactory ratio (respiratory quotient) of the carbon dioxide produced to the oxygen consumed was obtained for the alcohol, the apparatus was considered to be functioning properly.

The chamber rested on a platform, which was suspended by means of a pneumograph enabling the graphic registration of movements of the animal during the measured periods, by which means we selected quiet periods.

In the early part of our research an open-circuit respiration apparatus⁸ was used for determining the basal heat production of the marmot. As the animals began to hibernate, this was still used. We were unaware of the very minute quantities of carbon dioxide with which we would be dealing and did not realize the great disproportion between the large soda-lime recipients and the small quantities of gas to be absorbed. For this reason the earliest experiments were made with a technique that we do not recommend. Since the values are numerous and agree well and since we had long periods, we feel that these figures are reasonably correct. It is also to be noted that the early findings were substantiated by the more accurate techniques that followed as the research progressed.

⁷ Murrill, P., Journ. Am. Chem. Soc., 1898, **20**, p. 501.

⁸ A slight modification of the apparatus described and schematically outlined by Benedict, F. G., and C. G. Benedict, Carnegie Inst. Wash. Pub. No. 446, 1933, fig. 2, p. 32.

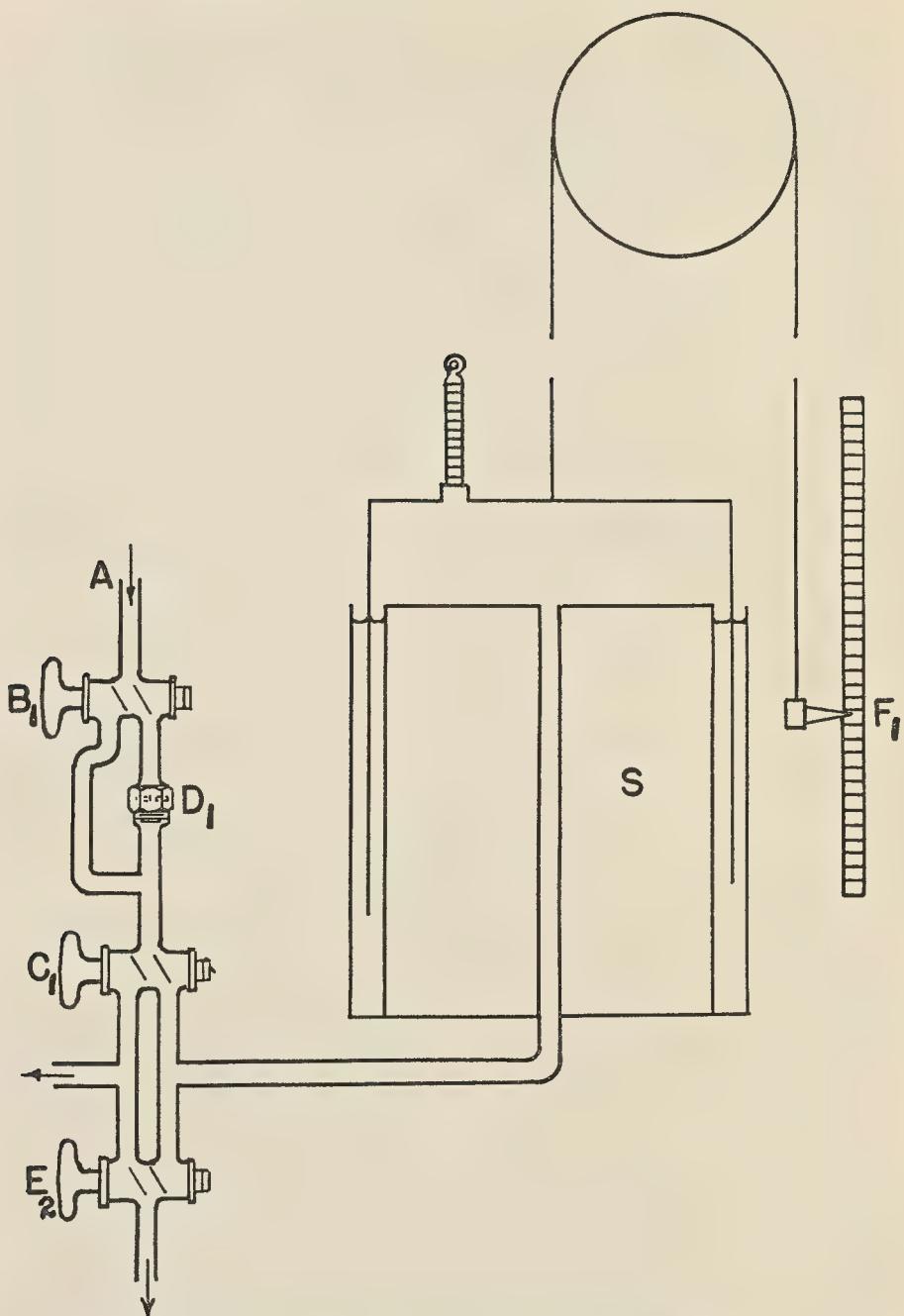


FIG. 3—DIAGRAM OF ALIQUOT SAMPLING DEVICE

Ventilating air (under a few millimeters water pressure) is supplied at A, disc D_1 allowing only a small portion of air to pass continuously into spirometer S. Stopcock B_1 by-passes the air current around disc D_1 , permitting the collection of the total outgoing air when very slow rates of ventilation are used. Stopcock C_1 permits direction of air into either spirometer S or a second spirometer, not shown. The volume of the collected air is read on scale F_1 . Stopcock E_2 is used to fill sampling pump and to empty spirometer.

METABOLISM MEASUREMENTS, HIBERNATING

The principles used to determine the gaseous exchange of the non-hibernating marmot were likewise applicable to the marmot in hibernation. Essentially the same apparatus was used, with proper modifications for the minute quantities of carbon dioxide and oxygen to be dealt with. For the experiments in the latter part of our studies the equipment shown in plate 2 (see page 12) was used. The ingoing air, supplied through tube A, is first dried by calcium chloride (not shown), and enters the chamber C (the details of which are shown in figure 1, page 14), with metallic top sealed by physicist's wax. The air is withdrawn by a small Collins blower (not shown), through tube B. The rate of ventilation is controlled by use of rotamessers, R₁ or R₂, which are adapted for different rates of ventilation. Since small quantities of carbon dioxide are produced in hibernation, it was necessary to have a constant ventilation rate, and long periods or a number of periods to insure correct measurements. A Haldane gas-analysis apparatus, E₁, connected directly to the ventilating air line at X, is used to determine periodically the percentage of carbon dioxide in the ventilating air. With this knowledge the rate of flow can be regulated to hold the carbon-dioxide percentage at the optimum level. When the animal is in deep hibernation, the total volume of outgoing air can be collected in the spirometer, S. Only one spirometer is shown in plate 2, but in practice two spirometers were used to enable consecutive periods. When the total volume of outgoing air was collected in the 2.5-liter spirometer, the stopcock, B₁ (see figure 3), was turned to by-pass the air current around the disc, D₁, and thus practically no resistance was offered. In experiments on waking marmots and in experiments where the animal had a relatively high heat production, the outgoing air was measured on a wet gas meter, M, which had a saturating water bottle, D, in line before it. In these cases aliquot samples were collected in the spirometer, S, as in the experiments on non-hibernating animals. In experiments when the marmots were waking, frequent control of the carbon-dioxide percentage was necessary to keep it at a measurable level. There were no determinations of the water vaporized by hibernating marmots.

As respiratory quotients appreciably below that characteristic of fat combustion have frequently been reported (many of these determined by techniques that are open to criticism), the need for a technique above reproach, to determine accurately the respiratory quotient during the transitory stages of entering hibernation and awakening from hibernation, as well as in the actual hibernating state, is evident. A chamber of sufficient size to contain a marmot has a relatively large volume. When one considers the slow ventilation rate required to keep the carbon-dioxide content at approximately one per cent, one realizes that there is a possibility for carbon dioxide to be absorbed by any moisture present, and for the carbon dioxide to diffuse through the rubber connections and rubber tubing always present. It is conceivable that any one of these factors, or a combination, might result in an inaccurate determination of the respiratory quotient of the expired air

of the animal. Various techniques were used before one was finally developed that was satisfactory to us.

The method finally adopted consisted of an arrangement to secure a sample directly from the chamber, practically without coming in contact with rubber tubing. It was also desirable to reduce the dead space from the chamber to the sampling tube to the smallest minimum possible. By introducing a capillary lead tube (of 0.9 mm. capillary) directly into the chamber (see plates 1 and 2 and figure 1) a true sample could be obtained. In order to maintain the ventilation rate constant in experiments with the marmot in deep hibernation, the outgoing-air-line was pinched off and the sample was

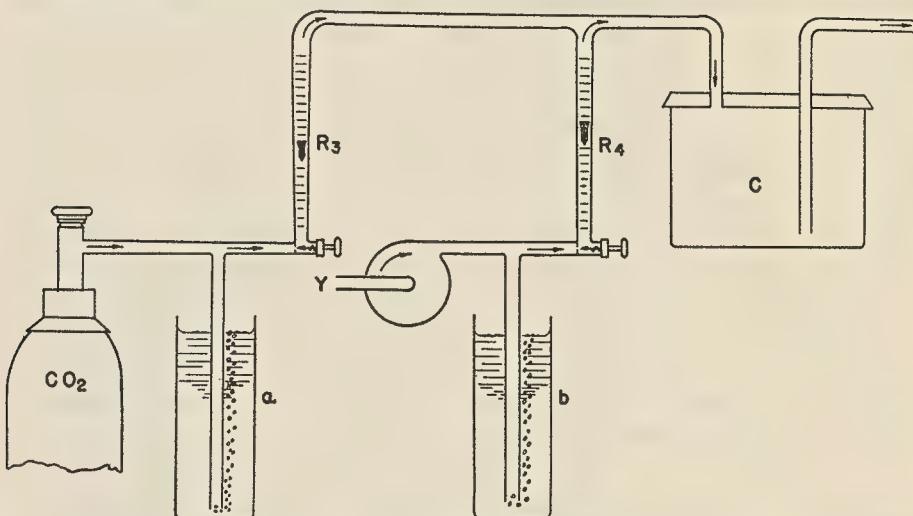


FIG. 4—METHOD OF OBTAINING CARBON-DIOXIDE MIXTURES FOR CARBON-DIOXIDE NARCOSIS

Carbon dioxide is introduced from cylinder, passes by a tee dipping in water bottle, a, to valve at base of rotamesser R₃. Oxygen or room air is supplied at Y, passes by tee dipping into water bottle, b, to valve at base of rotamesser R₄. The tees dipping in the water bottles, a and b, maintain constant pressure to the valves at the bases of rotamessers R₃ and R₄. By use of these valves a definite volume of each gas is supplied to the common lead, thus introducing a constant mixture of the gases to chamber C.

drawn at a rate to remove the air from the chamber at the rate removed by the blower just previously. The sample tube was filled once, and the air rejected to the room before it was filled with a sample for analysis. The samples were analyzed on the extremely precise Carpenter gas-analysis apparatus, by our expert gas analyst, Mr. George Lee. The gas-analysis apparatus was always checked either with outdoor air or a sample of air obtained by burning ethyl alcohol, in which the relation of the carbon-dioxide increment to the oxygen deficit is 0.667.

USE OF NEMBUTAL

A study of several marmots was made when the animals were injected with nembutal,⁹ and subsequently subjected to a cold environment. This barbit-

⁹ Pentobarbital Sodium, Abbott.

urate was injected into the peritoneum by means of a hypodermic syringe. The material used was the veterinary solution, containing 1 grain (64.8 mg.) per 1 c.c. The usual dosage was 1 c.c. of this solution per 1.8 kg. body weight.

CARBON-DIOXIDE NARCOSIS

Several marmots were studied under the effects of carbon-dioxide narcosis and cold as described by Dubois.¹⁰ This carbon-dioxide narcosis was carried out by placing the animal in a glass chamber (see plate 1) having a metallic cover sealed on with physicist's wax. A mixture of carbon dioxide and air, or in some cases carbon dioxide and oxygen, having the correct percentage, was supplied, with the use of rotamessers. (See figure 4.) By immersing a tee, connected with a tube from the cylinder, in a container of water (a), carbon dioxide under constant pressure was supplied to a regulating valve at base of flow meter, R₃. Either room air or oxygen was supplied at Y under constant pressure by a similar device (b) to valve at base of flow meter, R₄. The use of these two calibrated flow meters, in conjunction with needle valves, enabled an accurate mixture of gases in any desired proportion to be supplied for an indefinite period of time to the chamber (C). The total volume of gas supplied in this case was always one liter per minute:

¹⁰ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, pp. 246 et seq.

PHYSIOLOGY OF THE NON-HIBERNATING MARMOT

BODY WEIGHT CHANGES

To contribute to the knowledge of the physiology of the marmot and especially to establish normal baselines as regards its physiological functions, for comparison with these same functions when the animal is entering hibernation, is in hibernation, and is leaving hibernation, a study of the animal in the non-hibernating condition must precede that in the hibernating condition. Such a study should include the usual physiological measurements with, in our case, emphasis laid upon energetics and the accompanying respiration rate, heart rate, and rectal temperature, to determine whether these latter factors are correlated with the most important factor of all, namely, the heat production. As a part of the physiological picture and incidental to these salient studies of the heart rate, respiration rate, body temperature, and heat production, we made numerous other measurements. The first to be considered are the changes in body weight, these changes being of importance not alone of themselves but also because they serve as indices of the normality of the animal, whether adult or immature, the rate of gain, and whether there has been a successful adjustment to feeding under conditions of captivity and laboratory life.

Having a relatively large colony of marmots at the Nutrition Laboratory for several months gave an opportunity for study of transitions in their weights when under conditions of captivity and in both non-hibernating and hibernating conditions. Those transitions that deal with the non-hibernating animal are to be especially considered, for they are more or less comparable to the transitions noted with other warm-blooded animals. In its yearly life cycle the marmot undergoes great changes in weight due, on the one hand, to the excess feeding incidental to fattening prior to going into hibernation during the fall and, on the other hand, to the prolonged drafts upon body tissue during the months of hibernation. Ideally a study of these body weight changes could best be carried out if the animals were free, had available their normal food material, and were not distressed or upset by confinement. Many laboratory animals, such as rabbits, dogs, and geese, are quite unconcerned as a result of confinement. On the other hand, marmots are wild, rather ferocious animals, and anyone working with them can see instantly that many of them are distinctly disturbed by confinement and remain so throughout practically the entire period of captivity. However, in spite of this disturbance of body habits and functions our large mass of data dealing with the fluctuations in the body weights of these animals when in the non-hibernating condition furnish, when analyzed, most interesting results.

Thirty-one marmots were weighed at intervals of four or five days over

considerable lengths of time in the non-hibernating condition. Nine of these actually gained 28 per cent or more in weight in an extraordinarily short time, in no case over 60 days. Indeed, seven of these animals gained 44 per cent or more. Sixteen gained or lost 15 per cent or less, and four lost 20 per cent or more. Obviously those that had lost weight, no matter how extensively, are of interest only in indicating that they were, in all probability, profoundly disturbed by captivity or did not have the particular food to which they were accustomed. The striking fact is that nine gained 28 per cent or more in weight in a short time on the laboratory food furnished them. A group of fifteen animals purchased in one lot in September all gave every

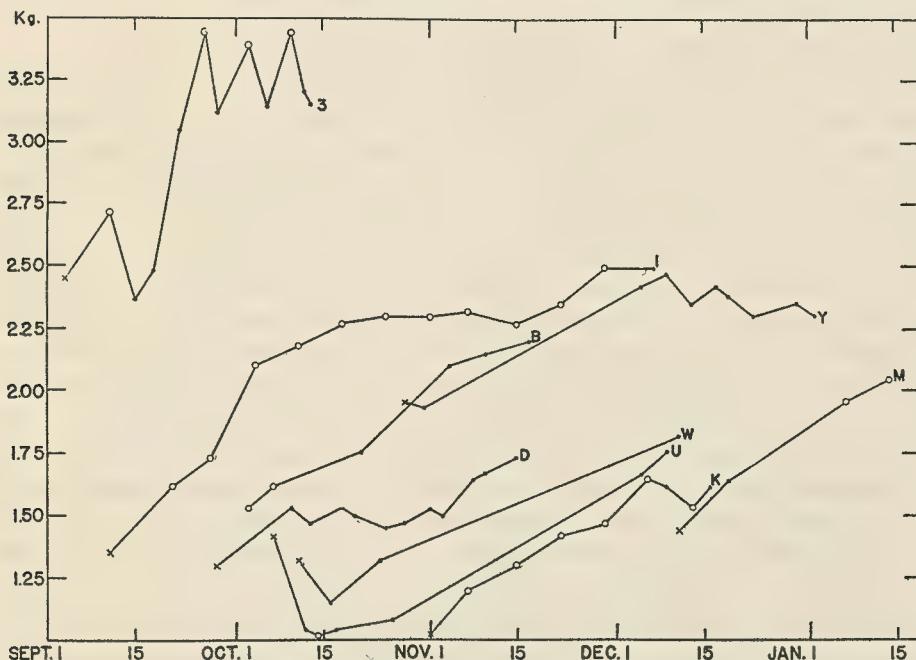


FIG. 5.—TRENDS OF THE BODY WEIGHTS OF NINE NON-HIBERNATING MARMOTS THAT GAINED 28 PER CENT OR MORE IN WEIGHT IN A SHORT TIME ON LABORATORY FOOD

The weight at time of arrival of the marmot at the Laboratory is indicated by a cross. The hollow circles indicate weights noted when the animals had had food before them, and the solid dots the weights after 24 hours of fasting.

evidence of being distinctly fat, *i.e.*, in well-rounded condition. Most of these made further gains on the laboratory feed, thus giving additional proof of a wild, captured animal adjusting itself rapidly to laboratory life.

The body weights of the nine marmots showing gains in weight have been plotted in figure 5 for the period from September 1 to January 15. The weights of these marmots were often recorded 24 hours after the withdrawal of food and just prior to the respiration experiment, for the special purpose of referring the metabolism to the body weight. On other occasions their weights were noted when food had been before them continually, and the animals may or may not have eaten just prior to the weighings. In figure 5

those weights that were taken when food had been present in the cage are indicated by hollow circles. Those weights recorded immediately upon the arrival of the marmot at the Laboratory are indicated by crosses. The solid dots represent weights obtained 24 hours after food had been withdrawn. Eight of these nine animals weighed initially under 2 kg., and two of them at one time or another weighed as low as 1 kg. All underwent marked and rapid gains in weight. This gain in weight may, in some cases, be attributed to normal growth, although the animals were probably all at least five months old. Obviously No. 3 with a weight of approximately 2.5 kg. on arrival was an adult animal. (See figure 5.) This marmot showed one of the most marked increases in weight of any, *i.e.*, from 2370 gm. on September 15 to 3440 gm. or somewhat over 1 kg. in ten days.

Our main concern in the size of these marmots is ultimately whether in our consideration of the measurements, particularly of the heat production, we are dealing with animals that are not fully adult. Strictly speaking, a study of the physiology of the marmot should include observations on the rate of growth, but as our main problem is to study hibernation and hibernation would normally not occur with these animals until they were several months old, we have to forego a complete study of the marmot's physiology at this early stage. Undoubtedly some of these animals were young. Of our entire group of 48 animals, the smallest weighed on arrival at the Laboratory just 1 kg. The next lowest weight was 1.19 kg. As these animals were without doubt born in late spring and their arrival at the Laboratory was almost invariably in September or October, they must have been about 5 or 6 months old. Approximately one-fourth of them weighed on arrival 1.7 kg. or under. Another group weighed at this time more nearly 2.5 kg. One individual, the largest in the entire series, weighed 4.98 kg. Fifteen animals obtained in one lot in September, 1937, were weighed as a group, and the average for the entire group was 3.1 kg. per animal. From these data we can conclude that the average 6-month old marmot would weigh not far from 1.5 kg., and that those a year older would weigh more nearly 2.5 to 3 kg. Little is known with regard to the precise period of gestation and the rate of growth of these animals, and *in lieu* of more precise data we shall simply assume that all our marmots were at least 6 months old. From the one instance when animals were born on the day of receipt of a pregnant female, April 30, 1935, we infer that this is not far from the normal birth date of these animals. (See page 8.)

One of the most striking points in this series of weights of non-hibernating marmots is the possibility of these animals, even one weighing 2.5 kg., having an enormous increase in weight on artificially prepared food and under conditions of captivity that disturbed some of the animals greatly.

Although most of the weighings were made when the animals had been fasting 24 hours, a number were made while they were feeding. The effect on the body weight of withholding of food for 24 hours was noted in a number of tests. On the average, the withholding of food for 24 hours results

in a loss of approximately 40 gm. If the withholding of food is continued for the next 24 hours it results, on the average, in an additional loss of not far from 70 gm. During the period of fasting from 48 to 72 hours the loss is not far from 60 gm., and in the eleven instances where the fasts were prolonged to from 72 to 96 hours, the further losses varied from 10 to 90 gm. and averaged not far from 50 gm. The body weight of the non-hibernating marmot will, therefore, decrease about 50 gm. during each 24 hours of early fasting. This is, to a certain extent, irrespective of the initial body weight, for a marmot of 2 kg. will show losses on the withholding of food essentially as great as will a 3-kg. animal.

A considerable number of our animals had their body weights determined over a period of several consecutive weeks when they were subjected to intermittent short fasts. The influence of these periodic fasts upon the body weights would be expected to be fairly severe. During each fast the animal had to draw upon its own tissue for maintenance for at least 36 hours. This would somewhat retard the rate of growth. The body weight curves of these marmots for the most part remained flat. The animals that were subjected to these 24- to 36-hour fasts every few days apparently, therefore, had already attained an adult weight (for the most part they weighed over 2.5 kg.) and the fasting periods were sufficient to hold them at these body weights. Another interpretation might be that their appetite for the artificially prepared food was not keen enough to induce them to eat enough of this food to more than compensate for the fasting drafts during this period, which otherwise would be a period when the animal with free food intake would be inclined to gain in weight in preparation for the winter sleep. As these records of body weights at this time were accompanied by metabolism studies, reference to the actual heat production of these animals under these conditions will be made subsequently. (See page 56.)

INSENSIBLE PERSPIRATION

The experience of the Nutrition Laboratory in associating the insensible perspiration or the insensible loss of body weight with the metabolism in the case of humans¹ and large animals² has led us to include in most of our inter-specific comparison studies a measurement, when feasible, of the insensible perspiration along with, if not simultaneous with, the gaseous metabolism. The changes in body weight of the marmot, especially during hibernation, have been a matter of interest for decades, owing to the contention of some writers that the animal could actually gain in weight during hibernation.

¹ Benedict, F. G., and H. F. Root, Arch. Intern. Med., 1926, **38**, p. 1; Benedict, F. G., and C. G. Benedict, Biochem. Zeitschr., 1927, **186**, p. 278; Benedict, F. G., The correlation between Perspiratio Insensibilis and total metabolism, Reprinted from Collection of articles dedicated to 75th birthday of Prof. I. P. Pawlow. Published from Inst. Expt. Med., Leningrad, 1924, p. 193.

² Benedict, F. G., and E. G. Ritzman, Carnegie Inst. Wash. Pub. No. 377, 1927, pp. 63 *et seq.*; Ritzman, E. G., and F. G. Benedict, Carnegie Inst. Wash. Pub. No. 494, 1938, pp. 74 *et seq.*

Therefore, the normal changes in body weight of these animals should be noted. Although the marmots, as a rule, were fairly quiet on the balance,

TABLE 1—*Insensible perspiration of non-hibernating marmots*

Marmot	Date	Body weight	Temperature		Insensible loss per kg. per 24 hours
			Dry bulb	Rectal	
C	1932		kg.	°C.	gm.
	Oct. 10	1.33	23.0	36.7	16.5
	" 11	1.30	20.6	37.1	13.3
	" 13	1.25	15.9	36.2	16.4
	" 14	1.18	15.4	36.8	15.0
	" 27	1.33	17.8	35.6	14.1
D	" 25	1.45	14.0	34.9	9.5
	Oct. 10	1.53	23.0	35.2	5.5
	" 11	1.52	21.0	36.5	14.9
	" 18	1.53	17.0	35.2	7.5
	" 20	1.50	17.2	35.6	8.4
	" 26	1.39	15.1	35.1	17.0
	Nov. 1	1.53	13.5	34.4	6.6
	" 8	1.64	13.3	34.5	5.2
	" 15	1.73	11.1	34.5	4.4
	Dec. 13	1.36	5.4	34.6	7.4
E	Oct. 12	2.64	18.1	35.2	4.8
	" 13	2.62	16.0	35.4	14.5
	" 18	2.58	18.8	35.3	6.3
	" 27	2.35	17.8	34.5	13.9
	Nov. 1	2.38	13.5	34.2	5.1
	" 3	2.41	11.5	34.8	4.9
	" 10	2.55	12.7	34.6	5.6
	" 26	2.64	10.0	33.8	6.8
	" 30	2.61	7.9	33.3	5.5
	Dec. 8	2.58	13.3	32.8	4.2
	" 14	2.55	5.6	33.5	15.1
	Oct. 14	2.27	14.3	36.3	8.7
	" 19	2.16	17.9	37.3	9.9
	" 25	2.04	14.1	35.2	8.3
	" 26	2.01	14.8	34.8	17.5
F	Nov. 2	1.93	12.6	34.8	6.5
	" 12	1.67	13.8	34.6	6.1
	" 23	1.42	6.2	33.5	5.8
	Dec. 1	1.64	9.5	34.3	5.8
	" 6	1.73	11.5	32.8	6.4
	1933				
G	Nov. 9	1.16	12.2	34.3	6.0
	" 16	1.11	12.3	37.6	7.1
S	" 18	1.08	11.4	36.1	6.7
	Dec. 7	1.19	15.2	35.1	5.8
Y	Jan. 10	3.35	21.6		13.8
	" 13	3.35	21.5		19.9
Y	Jan. 10	2.21	21.6		7.2
	" 13	2.31	30.7		16.1

¹ 48 hours without food; in all other instances 24 hours without food.

many experiments were lost due to the passage of urine and occasionally of feces. However, with seven animals sufficient uncontaminated data were obtained to justify reporting. These data, which are recorded in table 1,

represent two series, one in the fall of 1932 on animals C, D, E, F, and G, and one in January 1936 on animals S and Y. For the most part the measurements were made 24 hours after the last meal. The dry bulb temperature averaged 16° C., although in one experiment it was purposely raised to 31° C.

Marmot C, at an environmental temperature averaging not far from 18° C., showed generally an insensible perspiration per kilogram of body weight per 24 hours of about 15 gm. On the last day of observation, when the room temperature was 14°, the rectal temperature fell to the lowest level in this series (34.9°), and the insensible loss was down to 9.5 gm. This suggests that there is a correlation between rectal temperature and insensible perspiration. On two days of this series the animal was studied 48 hours after food, and the insensible loss was a little lower during the second 24 hours as can be seen from the data on October 11 and 14. With marmot D, in spite of considerable variations in environmental temperature from 5.4° on December 13 to 23° on October 10, the insensible loss remained reasonably uniform at not far from 6 gm., or essentially one-half that of marmot C which actually was somewhat lighter in weight. Thus, there is a striking difference in the insensible losses of these two animals. On the other hand, D had a somewhat lower rectal temperature than C. It is regretted that no basal metabolism measurements were made on these two animals, as such data might have assisted in interpreting the difference in their insensible perspiration.

With E, twice the size of the other two, the environmental temperature ranged from 5.6° to 18.8° C. and the insensible loss ranged from 3.9 to 6.8 gm., on the whole, a rather uniform loss. On the other hand, the rectal temperature of this animal was distinctly lower than the temperatures of the others, averaging under 34.5° C. In the one instance when the loss was measured in both the first and second 24 hours after food, that is, on October 12 and 13, the loss was slightly less on the second day, possibly complicated by a 2° lower environmental temperature in the latter case.

With F the environmental temperature ranged from 6.2° to 17.9° C., and the insensible loss from 5.8 to 9.9 gm. In this case the maximum insensible loss coincided with the highest environmental temperature and the lowest insensible loss with the lowest temperature. This animal showed pronounced rectal temperature fluctuations, the highest values occurring in October; thereafter there was a tendency for the temperature to decrease as the autumn months progressed, and the lowest temperatures were noted in December. Throughout these months there was a fall in body weight from 2.27 kg. on October 14 to as low as 1.42 kg. on November 23. During this time the animal must have been undergoing at least partial starvation, although food was before it at all times. In general the insensible loss per kilogram tended to decrease, as did the body weight. Toward the end of the series the animal had a body weight not significantly higher than that of C and D. Again the measurement made after 48 hours of fasting shows a slight decrease in the insensible perspiration per kilogram of body weight.

With G, the smallest weight animal in this series, the environmental tem-

perature was practically constant on all four days, averaging 13° C. The insensible perspiration averaged 6.4 gm., and the body temperature varied from 34.3° to 37.6° C.

In the 1936 series one of the largest marmots we had was S. This animal at an environmental temperature of 22° showed an insensible loss, on the average for four periods on January 10 and 11, of not far from 12 gm., a loss notably higher than any of the others except in the case of C. However, as S was a large animal, the value per kilogram represents a very large total insensible loss. The second marmot studied at about this time, Y, had a much smaller body weight, 2.2 kg. At a temperature of 21.6° C. the average insensible perspiration in three 1-hour periods was 7.2 gm. per kilogram, this being the same as the average for F but higher than the averages for all the other animals except C and S. When Y was subjected to an environmental temperature of 31° C. the increase in the loss was enormous, from 7.2 gm. at 21.6° to an average of 16.1 gm. at 31°. In other words, the insensible loss was more than doubled.

Most of these marmots showed substantially the same insensible loss per kilogram of body weight per 24 hours, but one at least (C) had a persistently high loss and S, even considering its size, had a very high value per kilogram. The reaction to environmental temperature was strikingly shown by more than a doubling of the insensible loss per kilogram with an increase of 10° C. in environmental temperature. Although we believed formerly that the careful measurement of the insensible perspiration was of value as a physiological record and that not infrequently a close correlation between the insensible perspiration and the metabolism is to be noted, it is clear from these observations that with the present technique at least there could be no thought of substituting insensible perspiration measurements for metabolism records. In those instances where the rectal temperature was low there was a tendency, certainly with C and F, for the insensible perspiration to be lower. This is not so strikingly shown by D and E and yet, conversely, with higher rectal temperatures there is usually a high insensible perspiration. Unfortunately in the 1936 series the rectal temperatures were not recorded, although undoubtedly the rectal temperature of Y on January 13 was high.

HEART RATE

The general unapproachableness of these marmots, their excitability, and viciousness made measurements of the heart rate not extremely satisfactory. The application of a stethoscope was out of the question, as this necessitated holding the animal. Based upon our experience with the cardiotachometer³ we made a number of tests to get some approximate idea of the heart rate of these animals. In our first attempt at securing the heart rate of this highly irritable, if not, indeed, ferocious animal, the marmot was so active that it had to be strapped to a board, a distinctly abnormal condition. The

³ Boas, E. P., Arch. Intern. Med., 1928, 41, p. 403; Boas, E. P., and E. F. Goldschmidt, *The heart rate*, Baltimore, 1932.

heart rate was counted every minute for nearly one hour. All the counts were made with the use of a cardiotachometer by counting the deflections of the galvanometer, which could be most accurately observed. The first count with this animal (U on October 21, 1935) was 200 beats per minute. The rate then gradually decreased until a fairly constant level was reached, the range being from 20 beats in 0.13 minute to 20 beats in 0.16 minute and the average 142 beats per minute, which is evidence of the high heart rate distinctly under duress.

On one occasion the electrodes were fastened to two flat boards and then placed at the bottom of the Abderhalden cage, and the marmot (No. 4, Nov. 18, 1936) stood for a number of minutes on these electrodes. Under these conditions several counts indicated a heart rate as low as 72 beats per minute. These were supported by four counts of 76 beats. It is, therefore, legitimate to say that the minimum heart rate of No. 4 could be considered to be not far from 73 to 75 beats per minute.

With No. 2 a different procedure was necessary. The electrodes were actually attached to the two front paws of the animal, being held there and taped on with adhesive tape. The struggle made by this particular animal in the attempt to remove the tape was such that no heart rates lower than 111 could be recorded. Following these observations, which were made in the morning, No. 2 was again studied in the afternoon, the electrodes being re-attached at 2.45 p.m. The animal was observed for an hour, but the heart rate never was below 110, which was the lowest rate observed in the morning. The highest afternoon rate, noted 10 minutes after the electrodes were attached, was 147, whereas in the morning the highest rate was 125.

Marmot No. 1 struggled to an unusual degree when the electrodes were put on, and for 10 minutes observations showed no rates below 143 beats per minute. The animal was left for several hours with the electrodes attached. At 11.30 p.m., when the building was quiet, the heart rate was again essentially 143 beats per minute, even though the animal was not approached as the heart rate apparatus was on a different floor from the animal. This is evidence that No. 1, undisturbed by the presence of the operator, had an uncommonly high heart rate. No. 1 was left all night with the electrodes in place, and the next morning at 9.50 a.m. the lowest heart rate was 118. Two hours later the lowest was 125 beats per minute. Thus the persistently high heart rate of No. 1 is confirmed.

The electrodes were similarly strapped onto No. 3 with adhesive tape. The data obtained on this marmot are summarized in table 2, as they represent characteristic values for an animal that was studied rather extensively. Emphasis is laid upon the variability in the heart rate of one and the same animal. When No. 3 was first studied, immediately after the electrodes were strapped on, the highest recorded value by this technique was 143. In the next 7 minutes the rate had settled to 100, and it fluctuated between that and 118 in the next 7 or 8 minutes. Following a period of quiet the animal was studied for about half an hour (*i.e.*, from 2.12 to 2.40 p.m.) when the

heart rate fluctuated from 75 to 125. The lowest rate in this series was reached at 2.33 p.m., namely, 75 per minute. Finally in the late afternoon with continued isolation the rate, although not so low as the 75 noted previously, had smaller fluctuations over a period of 18 minutes, ranging only from 80 to 95. The maximum values found with the other three animals not restrained were 154 with No. 1, 147 with No. 2, and 143 with No. 4. The minimum values, which are more important physiologically as they represent more nearly the basal level or physical repose, were 118 with No. 1, 110 with No. 2, 75 with No. 3, and 68 with No. 4.

TABLE 2—Heart rate of non-hibernating marmot No. 3
(Kept and measured at 28° C., Nov. 18, 1936)

Time	Beats counted		Rate per minute	Time	Beats counted		Rate per minute
	No.	Time			No.	Time	
<i>a.m.</i>							
11.32	20	0.14	143	2.12	20	0.235	85
11.33½	20	.15	133	2.12½	20	.225	89
11.34½	20	.14	143	2.13	20	.21	93
11.36	20	.155	129	2.13½	20	.22	89
11.37	20	.165	121	2.15	20	.16	125
11.38	20	.16	125	2.15½	20	.19	105
11.39	20	.20	100	2.16	20	.205	98
11.39½	20	.19	105	2.18	100	.98	102
11.40	20	.18	111	2.19	100	1.03	97
11.40½	20	.18	111	2.21	100	1.06	94
11.41	20	.175	114	2.23	100	1.10	91
11.42	20	.185	108	2.24½	100	1.08	93
11.42½	20	.18	111	2.26	100	1.09	92
11.43	20	.17	118	2.33	100	1.34	75
11.44	37	.32	116	2.35	100	1.25	80
11.45½	100	.92	109	2.37	100	.99	101
11.46½	100	.89	112	2.38½	100	.955	105
			2.40	100	1.015	99	
			4.05	100	1.05	95	
			4.07	100	1.09	92	
			4.09	100	1.13	89	
			4.11	100	1.09	92	
			4.12½	100	1.14	88	
			4.15	100	1.145	87	
			4.20	100	1.255	80	
			4.23	100	1.07	93	

Although the animal was isolated from the observer during these observations, the data obtained are not ideal. They do show, however, that the marmot under duress and restraint may have a heart rate of 140 and over, that after excitement the rate may be as high as 200, and that the lowest rate observed was 68 per minute with No. 4. A general average value that might be considered to be characteristic of the non-hibernating marmot in reasonable repose would be 80 beats per minute. This is of special significance for further comparison later with the rates of marmots that are hibernating and coming out of hibernation.

RESPIRATION RATE

Any attachment to the body to record graphically the respiration rate of the irritable marmot is out of the question. The movements of the chest or

abdominal wall are the only available indices of normal respiration rate. Records of such movements necessitated the close proximity of the observer, and this in turn reacted on the excitability of the animal. Therefore, from the large number of observations made the inference is drawn that only when there are a number of values that support each other, can these be taken as uncontaminated respiration rates. The first observations were made in October 1932, at 20° C., with C, D, and F, 24 hours after food. The marmots were suspended upon a balance for the determination of the insensible perspiration. Under these conditions the respiratory movements of C showed minimum rates of 11 and 15 per minute; of D, 15 and 10 per minute; and of F, 17 and 10 per minute. Marmot C, when fasting 51 hours, had an average rate of more nearly 23 per minute.

Marmot L, which was dug out of the ground in hibernation, arrived at the Laboratory on November 14, 1934, and apparently did not eat anything after that date. On November 28 when the animal was seemingly undisturbed, the rate was 24 respirations per minute. The other rates ranged more nearly from 60 to 70 per minute. Observations were made every few days from December 1934 to April 1935 on M, when the marmot had food available, thus preventing any appearance of hibernation. During this time the environmental temperature fluctuated considerably. This marmot had a number of respiration rates in the neighborhood of 25 per minute, but there were several indications of its being "sleepy." With both L and M there was a general tendency for the respiration rate to be higher at the somewhat higher temperature.

The respiration rate of marmot U was determined on October 21, 1935, but under distinctly abnormal conditions, for the animal was strapped to a board. Fifteen minutes after being attached to the board, it showed a respiration rate of 91 per minute, and twenty minutes later the count had dropped off to an average of not far from 36 per minute, which is surprisingly low when one considers the restraint that accompanied it. Three other animals studied at this time but not restrained showed high rates of 70 and over.

A special study in successive 1-minute periods for 10 minutes was made on September 30, 1936, at 28° C., with Nos. 5 and 10. These two marmots had normal minimum respiration rates of approximately 25 to 30, as shown by the data in table 3. These observations furnish an admirable index of the variability that may be expected in the respiration rate of the marmot under normal, quiet conditions and the transient effect of excitation. Thus, with No. 5 the rate varied from 30 to a maximum of 69, when the animal had been startled. The great change in the rate from minute to minute is clearly shown by this series of counts. On the other hand, with No. 10 that remained very quiet, lying on its side, the rates throughout the entire 10 minutes ranged only from 25 to 30, an extraordinary degree of regularity.

Marmots 9 and 10 were studied almost daily from December 3, 1936, to January 18, 1937, at an average temperature of about 12° C. The observations on the respiration rates were complicated by the fact that the animals were sleeping part of the time and for the most part were in a sluggish condi-

tion. In general, however, the respiration rate of No. 9, when not sleepy, was at a minimum level of about 20. On January 19 and 20 the environmental temperature was raised to 30° C., and the respiration rate of No. 9 increased to approximately 40 per minute. Marmot No. 10 had a minimum rate definitely higher than the 20 per minute of No. 9, its basal value being about 26 per minute. When the temperature was raised to 30° C., the respiration rate of No. 10 likewise approached 40 per minute.

In general it may be concluded that the non-hibernating marmot has maximum respiration rates in the vicinity of 100 per minute when agitated but that when it is quiet and at moderate environmental temperatures of from 10° to 25° C. the rate is not far from 25 to 30 per minute. At environmental temperatures of 30° C. the respiration rate is almost doubled. This increased rate, in view of the subcutaneous fat layers and heavy pelage, may be interpreted as approximating the "panting" of the dog as an aid to heat loss.

TABLE 3—*Variability of respiration rate of non-hibernating marmot
(September 30, 1936, at 28° C.)*

Minute	Rate per minute	
	Animal 5	Animal 10
1st	37	27
2nd	32	25
3rd	32	29
4th	31	30
5th	30	30
6th	169	29
7th	52	27
8th	65	25
9th	40	26
10th	42	26

¹ Animal startled.

RECTAL TEMPERATURE

Although the marmot when non-hibernating may be classified as a warm-blooded animal, the lability of the body temperature of animals that are prone to hibernate is such that a more than ordinarily careful study of the normal non-hibernating temperature is justifiable. The marmot may be considered to be heterothermic, but even among the other warm-blooded animals that do not hibernate there are some that have variable body temperatures, especially at the low environmental temperatures associated with hibernation. The mouse, for example, has an extraordinarily labile body temperature.⁴ Indeed, Aszódi⁵ noted with mice body temperatures that were definitely in the range of those characteristic of hibernating animals. In order to study the rectal temperature of the marmot, obviously this irritable animal must be restrained and hence under considerable tension, which undoubtedly has an influence upon the temperature itself. There

⁴ Benedict, F. G., and R. C. Lee, *Annal. de Physiol.*, 1936, **12**, p. 983.

⁵ Aszódi, Z., *Biochem. Zeitschr.*, 1921, **113**, p. 70.

was always a struggle to catch the animal, even when it was in a chamber, and it was frequently held by a noose and with gloved hands. The rectal temperatures recorded under such conditions may rarely be directly comparable with those obtained on more placid and cooperative animals that are ordinarily used in laboratory work. Hence it may be stated at the outset that all rectal temperatures recorded on the non-hibernating marmot may be considered to be too high, rather than too low.

Influence of struggle and excitation. The specific effect of activity was studied on several occasions when the marmots were purposely agitated by struggle or by chasing them around the cage or even the room. The initial rectal temperature, somewhat high due to the struggle in capturing the animal and inserting the thermometer, was further elevated as a result of the excessive struggle. Two series of such experiments were made. On one occasion No. 23 was chased for six minutes around the room. The rectal temperature prior to this test, but taken after some struggle, was 37.2° C. After the 6-minute chase around the room, the temperature continually rose until the third minute, when it reached 37.9° C. No. 26 was given the same treatment. At the start there was considerable struggle to catch the animal and a little struggle during the measurement. The rectal temperature was 37.4°. After about 6 minutes of enforced activity, the temperature rose rapidly for 4 minutes to 38.3° C. With S the initial temperature was 38.6°, following, however, considerable struggle. After S was chased around the room, its temperature rose to 39.7° C. With U the temperature prior to struggle was 38.2° and after chase 39.1° C. Marmot R had a relatively low temperature, even though there was some struggle prior to taking the initial reading which was 35.3°; after it was chased, its temperature was 37.1° C. No. 2 increased its temperature from 36.1° to 39.2° and No. 3 from 36.6° to 38.1° C. It is thus clear that excitability and struggle react rapidly on the marmot, and that a rise of 1° or more in the rectal temperature within a few minutes may be expected when the struggle is rather considerable. This emphasizes again the fact that since inevitably some struggle was necessary to catch the animal and hold it in restraint, all our temperature records may be considered to be somewhat high rather than too low, although in our averages we have attempted to use only those records that were not obviously affected by struggle.

Temperature gradient inside the body and time element. As the marmot so obviously has a labile temperature, rapidly affected by struggle or excitation, the question as to the actual procedure for determining properly and correctly the temperature naturally arose. To what depth must a thermometer be inserted to measure and insure normal, true body temperature uninfluenced by the temperature of the environment and how long after insertion of the thermometer must one wait to obtain the true temperature? The time element depends on the nature of the thermometer, whether it is mercurial or a thermo-junction. Body temperature measurements are complicated, however, by the fact that marmots are restless and the thermometer

in the rectum is an irritation. A number of studies led to the practice of inserting the thermometer to a depth of 140 mm., and keeping it in place until the deflections on the galvanometer had attained constancy or the mercury had risen to a constant point. Usually this took place in from $2\frac{1}{2}$ to 3 minutes, when the true body temperature could be obtained.

Influence of environmental temperature. In view of the fact that the marmot is so profoundly affected by environmental temperature, particularly low temperatures such as to induce hibernation, and in view of the already indicated lability of its body temperature, obviously a study of the effect of environmental temperature upon the rectal temperature of the non-hibernating animal was important. These studies were of two different types. In the first type the animals were studied over a period of several days during which they were maintained in the non-hibernating condition at an environmental temperature averaging not far from 16° C. Four of these animals, D, H, I, and K, had average rectal temperatures of 35.1°, 35.7°, 36.5°, and 36.5° C. If an average of these few values is permissible, the average temperature of the non-hibernating marmot at environmental temperatures of about 16° can be stated to be 36.0° C. The second type of experiment was to superimpose considerable variability in environmental temperature, particularly high rather than low temperatures. Many observations were made at 28° and particularly when the marmot had been maintained at this temperature for some time. Under these conditions the rectal temperature was usually somewhat above 36° C. The animals were studied either directly off food or 24 hours after food had been withdrawn. The general tendency was for the rectal temperature at 28° C. to be approximately 37° or about one degree higher than when the marmots were measured at 16° C. A few animals were studied when they had been kept at a relatively high temperature for the marmot, that is, at 30° C. These had in general a high rectal temperature in the neighborhood of 38° C. When the marmots had been kept at 34° for several weeks they had rectal temperatures approaching 39° C. Rather sudden transitions in temperature were made with another group, the marmots being measured at one temperature and then suddenly subjected for two hours or so to a much lower temperature. When the animals had been living at 27° and were then subjected to six 25-minute periods at 16°, the rectal temperatures fell usually somewhat below that of animals continually maintained at 16° C. On the contrary, when the marmots had been maintained for some time at 16° and were then measured after about two hours at 28°, the rectal temperatures were usually somewhat higher, not strikingly so but still slightly higher than the value noted at the environment of 16°. However, when the animals had been kept at over 30° for any length of time, the rectal temperatures were always between 38° and 39° C.

Variability of normal rectal temperature. Although in general when the marmots had been living at 16° under normal garage conditions the average rectal temperature was 36°, innumerable instances were found where ani-

mals, living under these conditions and at a time of year when they could not possibly have been approaching hibernation, had normal rectal temperatures as low as 34.3° C. Hence a marmot having a body temperature of 34.3° C. may not in any sense be considered abnormal. Indeed, although in general in an environment at 16° the average normal rectal temperature of the non-hibernating marmot is 36° C., a temperature as low as 33° C. would not be considered to be below the normal limit.

The effect of short fasts. As the most important use of the rectal temperature measurements was for comparison finally with the metabolism, both in the hibernating and non-hibernating conditions, and as one of the prerequisites for determining the basal metabolism was a short period of fasting, the influence of short fasts at 28° upon the rectal temperature was noted. The fasts were usually from 24 to 96 hours. Six marmots showed no significant effect of fasts lasting up to 96 hours. Hence, other things being equal, the rectal temperature of the marmot after 96 hours of fasting may be considered to be normal.

Body temperature observations during refeeding. In the spring after some of these animals had been through the drastic winter season, fasting and hibernating, they were refed, kept at 28°, and measured at this temperature. It was noted that in all cases the rectal temperature, which in the fall prior to hibernation (as stated on page 34) would be in the neighborhood of 37°, was now more nearly 38.0° C. The lowest recorded in the relatively few experiments made was, as a matter of fact, 37.4° C. However, the metabolic level (as will be seen later in the discussion of the gaseous metabolism—page 63) was higher in the spring in the refeeding period than in the pre-hibernation period. Although the rectal temperature may have been slightly affected by greater activity, it is hardly conceivable that the entire picture would be so affected by activity, although the heat production was measurably higher than that noted earlier in the fall.

General conclusions regarding body temperature. The rectal temperature of the non-hibernating marmot has definitely different values. When it is living at an environmental temperature, for the most part, of 16°, the rectal temperature is, on the average, 36° C. When it is maintained at 28° C., its body temperature averages 37° C. If the animals are subjected to environmental temperatures of 30° to 35° for several weeks, the rectal temperatures average 38.5° C. In other words, the temperature of the marmot is profoundly affected by the temperature in which it sojourns. These normal average rectal temperatures are entirely outside the range of temperatures incidental to the marmot's going into hibernation. In none of the instances included in these averages recorded above were the animals approaching or undergoing the initial stages of hibernation. These stages are so pronounced that they are unmistakable and will be discussed subsequently (pages 98 *et seq.*) under the head of the transition period from non-hibernation into hibernation.

NITROGEN EXCRETION OF THE NON-FASTING MARMOT

As our main study of the marmot was to be two-fold, one on the influence of prolonged fasting and the other on the influence of hibernation, few observations were made with the animal on food. The basal metabolism of the marmot was carefully studied 24 hours after food. Sufficient experience

TABLE 4—*Urinary nitrogen excretion of non-fasting marmots*

Marmot	Date	Body weight	Length of period of collection		Nitrogen excretion per 24 hours	
			days	hrs.	Total	Per kg.
E	Dec. 8-12	2.58	4		1.42	0.55
F	Dec. 8-12	1.84	4		1.13	.61
G	Dec. 10-12	1.13	2		.84	.75
H	Sept. 22-26 " 26-30 " 30-Oct. 5	2.90 2.87 2.94	3 3 4	23 18 22	1.64 1.37 1.24	.57 .48 .42
I	Sept. 20-23 " 23-27 " 27-Oct. 2 Oct. 2- " 5	1.65 1.70 1.90 2.10	2 4 5 3	20 3 18	1.96 1.74 1.44 1.85	1.19 1.02 .76 .88
M	Dec. 21-25 " 25-29 " 29-31 " 31-Jan. 2 Jan. 2- " 5 " 5- " 7 " 7- " 10 " 10- " 12 " 12- " 15 " 15- " 16 Feb. 9-16 " 16-23 " 23-March 2 March 2- " 9 " 9- " 16 " 16- " 21 " 21- " 23 March 28-30 " 30-April 5 April 5- " 10	1.72 1.80 1.82 1.86 1.92 1.94 1.96 2.06 2.20 2.22 2.02 2.16 2.20 2.27 2.30 2.33 2.35 2.41 2.45 2.48	4 3 1 2 3 2 3 1 2 1 6 7 7 6 7 5 1 1 6 6 4	7 19 20 2 2 3 9 14 20 2 21 7 1 1 20 2 2 23	.84 .84 1.40 1.26 1.69 1.85 1.25 1.60 1.28 1.64 .59 .66 .39 .42 .76 .93 .71 .92 .64 .99	.49 .47 .77 .68 .88 .95 .64 .78 .58 .74 .29 .31 .18 .19 .33 .40 .30 .38 .26 .40

in handling the quantitative feeding of these animals was not had at that time. They were, therefore, simply given food *ad libitum*, and no record was kept of exactly how much was eaten. In the fall of 1932 and 1933 and the winter and spring of 1935, however, measurements were made of the urinary nitrogen excretion of six animals while on food. The results are shown in table 4. Naturally these animals, ferocious and wild as they are, were

greatly influenced by their changed environment and diet. Hence these figures can be looked upon only as approximate measures. Furthermore, as the marmots represented various stages of maturity, one could not know whether the liberal feeding was accompanied by actual deposits of fat or deposits of nitrogenous tissue. The animals can be divided roughly into two classes, those that had reached their full growth and those that were still growing. These animals varied in weight considerably. Obviously E and H were adults. The trend of the body weights of I and M suggest that these marmots were definitely growing. The single weight records for F and G indicate that the latter was distinctly immature and F had not yet reached its full growth. The marmots were placed in Abderhalden metabolism cages, and the urine was collected for periods ranging from approximately 1 to 7 days. The results of the nitrogen determinations are reported on the basis of the total nitrogen excretion per 24 hours and likewise the nitrogen excretion per kilogram of body weight per 24 hours.

The amounts of total nitrogen excretion from period to period agree reasonably well. There is a striking difference in the amounts per kilogram per 24 hours with relation to the size of the animal. The smallest animal, G, had an excretion of 0.75 gm. on this basis, whereas H excreted nearer 0.50 gm., on the average. Animal I has high values ranging from 1.19 to 0.76 gm. and averaging twice that of H. Animal I was caught on August 15 and brought to the Laboratory on September 11, weighing on arrival 1.36 kg. This is proof that it was immature, and it is possible that some of this high nitrogen metabolism may have been due to the excessive feeding of this animal while gaining weight. As a matter of fact, the 4 periods of nitrogen measurement extended only from September 20 to October 5, and during this time the animal gained in weight essentially 0.5 kg., which indicates a rapid period of growth. Marmot G, which also was immature as suggested by its weight, likewise had a high nitrogen output per kilogram when compared with that of the other animals.

These values are, as stated above, only approximate and are of chief significance with reference to the rather plentiful data secured during prolonged fasting and hibernation. (See page 153.) During such times as well as when the marmot is on food the amount of urine is extremely small, and the values obtained during hibernation are likewise uncertain save that they do cover long periods.

Marmot M was received at the Laboratory on December 11, when it weighed 1.44 kg. Being dug out at that late date, probably it had already entered hibernation, but from December 12 until the end of the urine collection periods in April it did not hibernate, although it was subjected to periods of fasting in an attempt to induce hibernation. The animal grew rapidly as shown by the body weight increase from 1.64 kg. on December 19 to 2.20 kg. on January 15, that is, half a kilogram in less than one month. There was then a 2-week period of fasting, in which the body weight dropped back to

1.80 kg. on February 1. On February 23 the animal had increased in weight to 2.16 kg., and a month and a half later, on April 11, it weighed 2.50 kg. Thus there were two periods in which M gained half a kilogram or more, the first time in one month, the second time in $1\frac{1}{2}$ months. The periods of collection were usually from $1\frac{1}{2}$ to 3 or 4 days. Six of the periods were from 6 to 7 days long. The total nitrogen output during December and January averaged 1.4 gm. per 24 hours, the nitrogen output per kilogram averaged 0.7 gm., and the average body weight was not far from 2 kg. In the second half of the experiment, *i.e.*, from February 9 to the end, the total daily output decreased to an average of 0.7 gm. or half that in the first series, and there was a similar decrease in the nitrogen output per kilogram per 24 hours to about 0.3 gm.

In spite of the uncertainty regarding the quantitative food intake of these marmots, it seems clear that during the first series of observations on M we were dealing with an immature animal in a period of growth. As no nitrogen balance experiment was made, it is impossible to state whether there was a significant addition of nitrogen to the body at this time or not. From the results in the second series which, although accompanied by an increase in weight, show a much lower nitrogen output, one might infer that this increase was actually fat and that the laying on of nitrogenous tissue had slowed up, if not ceased. In the second stage, the nitrogen excretion of M per kilogram was as low as, if not a little lower than the excretions of the other five animals. During what we assume to be the actual stage of growth from December 21 to January 16 the nitrogen excretion of M per kilogram was definitely higher than the excretions of three of the other animals but somewhat lower than those of the presumably young animals I and G.

Although the evidence is by no means perfectly clear, nevertheless one can infer that the nitrogen output of an adult non-hibernating marmot, when normally fed under laboratory conditions, would be not far from 0.5 gm. per kilogram per 24 hours. During the period of growth when there is a larger food intake to provide for the deposits of nitrogenous tissue, there is a larger nitrogen excretion which may be in the neighborhood of 0.7 to 1 gm. per kilogram per 24 hours. These values, although approximate, are of chief significance for reference to the measurements during complete hibernation, as will be mentioned later on.

In connection with the studies of the nitrogen output during prolonged fasting and during hibernation, a few collections of urine made when the animals were on feed were analyzed more fully. From these fragmentary data further conclusions have been drawn by our colleague, Dr. T. M. Carpenter, with regard to the partition of the nitrogen excretion of the normal marmot when on feed. These conclusions, however, have more pertinent bearing on the discussion of the partition of the urinary nitrogen under the two conditions of prolonged fasting and hibernation and consideration of the same, therefore, will be deferred until that point.

GASEOUS METABOLISM

Our study of the gaseous metabolism of the marmot has two phases. In the first place, it deals with the gaseous output *per se*, that is, the carbon-dioxide production and the oxygen consumption with the special purpose of indicating not only the character of the material burned in the body when the animal has been subjected to a short fast but especially the rate at which the character of the material burned will change with the transition from food to fasting. As the marmot is fed primarily a carbohydrate diet, one would expect to find that when it is studied on feed its respiratory quotient would approximate 1.00, and in consideration of the fact that the marmot shows a distinct tendency to lay on flesh, namely, to convert carbohydrate into fat, one would expect that if liberal amounts of the normally carbohydrate-rich feed were eaten, the respiratory quotient at times at least would be above 1.00. The second phase of the gaseous metabolism study deals with the energy relations. The analysis of the respiratory quotient data indicating the character of the material burned is of only secondary interest in our major thesis, and the greatest emphasis should be laid upon the energy relations. Hence in the last analysis the chief use of the gaseous metabolism measurements is as a basis for the calculation of the heat production. This is particularly true in the case of the marmot, especially when hibernating, under which conditions direct calorimetry is practically impossible, owing to the small amounts of heat produced.

In the presentation of our gaseous metabolism measurements on marmots the carbon-dioxide production and the oxygen consumption will not be reported as such, but the results will be presented in terms of the respiratory quotient and the heat production, the latter expressed on the basis of 24 hours. The actual lengths of experimental periods differ widely in different laboratories, determined in part by the degree of repose or restlessness of the animal and in part by the different techniques used. It has been argued that it is illogical to express the heat production per 24 hours based upon a 5-minute period of measurement, to state an extreme case. To alter at this time the basis for reporting metabolism measurements in discussing phenomena that are of themselves most challenging would simply obscure the findings, and leave our readers confused. For these reasons the expression of the heat production per 24 hours is retained throughout this book, even though the metabolism was measured at times in only 10- to 15-minute periods.

Because of the large number of marmots studied, because of their differences in weight, and particularly because of the tremendous variations in metabolism between the non-hibernating and the hibernating state, the presentation of the data in the simple terms of total measured carbon-dioxide production and oxygen consumption is wholly inadequate. In view of the extensive metabolic comparisons that are to be made subsequently, we have resorted in the tabular presentation of the material to the time-honored methods of presenting this type of data, namely, as the total heat production

and the heat production per kilogram of body weight and per $10w^{\frac{3}{4}}$. This latter form of presentation is considered to represent sufficiently closely the heat production per unit of surface area, as the calculation of the true surface area is highly uncertain. For many years in its studies of numerous animals the Nutrition Laboratory has attempted to find correlations between body size and metabolism that will more nearly indicate uniformity in the heat production of animals of various sizes. It is clearly recognized that the heat production per kilogram of body weight is not equal with different animals in the same species, for the smaller the animal the higher is the heat production per kilogram. Furthermore, the Nutrition Laboratory's extensive series of measurements on large and small animals shows that, contrary to common belief, the heat production per square meter of surface area is not uniform. At the moment of writing, the protocols of all the animal experiments made at the Nutrition Laboratory are being resolved in the attempt to note whether there are any correlations between various physiological factors and the heat production. As striking reversals of the supposed picture presented by the metabolism of these animals are noted in the analysis of the marmot data, we are of the opinion (although in entire disagreement with the belief that the best method of comparing the heat production of animals of different sizes is upon the basis of the heat production per unit of surface area) that it is unwise to attempt at this time to introduce any other method of comparison, on the ground that for those who are wont to think in terms of surface area a different method of comparison would confuse an already confusing and wholly unexpected picture. Hence purely in order to minimize any further complication of the picture we retain the time-honored expressions of the heat production per kilogram of body weight and per square meter of body surface or, more precisely, per $10w^{\frac{3}{4}}$, and unless otherwise stated the values given in the text discussion will refer to the 24-hour heat production per $10w^{\frac{3}{4}}$. The need of some method of expression for comparative purposes other than that of the total heat production is obvious, when it is noted that not only must the same animal be compared at different weights and different animals at different weights but more particularly the same animal must be compared when hibernating and non-hibernating, under which conditions there may again be widely different weight levels.

PREREQUISITES FOR BASAL METABOLISM MEASUREMENTS

In any study designed to compare the measurements of the basal heat production of one and the same animal and different animals one should carefully take into account the factors known to affect the basal metabolism and rule these out. In the case of the non-hibernating marmot these factors are primarily digestive activity, muscular activity, and environmental temperatures outside the zone of thermic neutrality. In addition, the possible influence of such factors as too short a period of adaptation to laboratory conditions after capture, the time of day, and the level of the body temperature should be taken into consideration. Before comparing the results of our

basal metabolism measurements on these marmots, therefore, we should first discuss the prerequisites for such measurements.

The post-absorptive state, as indicated by a study of the respiratory quotient

The primary object of studying the respiratory quotient was to note at what time after food had been removed the marmot could be considered to be in the post-absorptive state, a state prerequisite for basal metabolism measurements. There are two indices normally used with animals to prove this point, namely, the level of the respiratory quotient and the level of the heat production, of which the latter will be considered subsequently. Generally it is assumed that after withdrawal of food the respiratory quotient reaches rather rapidly the level of fat combustion, that is, about 0.70. This is the criterion of the arrival of the post-absorptive state that has been adopted for geese, rats, mice, and most other warm-blooded animals. With man it is commonly assumed that the post-absorptive state (when there has not been a draft upon the glycogen reserves) obtains at 12 hours after food is withdrawn, even though the respiratory quotient is much higher than 0.70, indeed as high as 0.82. The exact level of the quotient in the post-absorptive state depends on the character and amount of the previous diet. Even with humans the quotient under post-absorptive conditions can be greatly altered. Perhaps the most striking case is that of the late Dr. Reed Rockwood in Boothby's laboratory who, following a period of surcharging with carbohydrate, actually had a quotient 12 hours after food of 1.00.⁶ The relationship between this 12-hour quotient and the glycogen deposit is probably as yet not clear. In fact, there is a distinct movement at the Nutrition Laboratory to emphasize the importance of using the respiratory quotient after the withdrawal of food for a certain time as a measure of the glycogen reserve. Experiments with animals all point to the feasibility and importance of such use.

With the marmot the situation is complicated by the fact that it is impossible to control the amount of food eaten. Food may be offered, but the animal may not eat, and an experimental program may be entirely spoiled by the lack of desire of the animal to eat. Our experiments, therefore, had to be made in large part upon marmots whose food intake was unknown quantitatively. In a few experiments known amounts of the laboratory mash⁷ (usually 50 gm.) were eaten by the marmots rather rapidly. The animals (kept at 28° C.) were then frequently removed from their feed cages and studied shortly afterwards in the respiration chamber, and subjected to continued fasting, the longest fast being 103 hours. This study was made in an effort to determine whether 24 hours' withdrawal of food would give a condition that may be considered basal. Eight marmots were thus studied, and it was found that at the end of 24 hours after this known amount

⁶ Personal communication from Dr. W. M. Boothby.

⁷ Made from canned corn and yellow corn meal.

of food was eaten the respiratory quotient had not reached the fat level. In only one case was it as low as 0.75. In the second 24 hours, that is, 48 hours after the withdrawal of food, the quotients were at the fat level⁸ with one exception, that of No. 7 which was as high as 0.78.

From this series of controlled food experiments it can be inferred that to insure the appearance of a fat quotient the marmot must be studied at least 48 hours after food is withdrawn. To avoid any misunderstanding later on, however, it should be stated that most of the basal metabolism measurements made by us on these marmots were preceded by only 24 hours of *enforced* fasting, although it is not known exactly how long the animals had been fasting before this. The actual period of fasting may have been and probably was usually somewhat longer than the 24 hours elapsing between the removal of the food and the metabolism measurement. Under these conditions it has been conclusively proved (see p. 45) that the heat production was not decreased by a further 24 hours of fasting. Hence the post-absorptive heat production can be said to be essentially independent of the level of the respiratory quotient. This practice of an enforced 24-hour fast was followed when it was proved that many of our marmots showed a fat quotient after 24 hours' withholding of food.

In these experiments with known amounts of food it happened in four instances that the respiratory quotient was determined within two to seven hours after food, at which times quotients as high as 0.88, 0.87, 0.86, and 0.80 were noted. In this same series of experiments two animals, not measured immediately after food, had quotients of 0.91 and 0.87 24 hours afterwards. At the 48th hour all but one had quotients of 0.75 or below, one showing 0.78, and at 72 hours all showed values well within the range of so-called fat quotients, which continued to the 96th hour.

In another experiment the immediate reaction to food was clearly indicated. On January 18, 1935, marmot M (weight 1.99 kg.) was studied for three periods, 47 hours after food, and the respiratory quotient for the three periods was 0.73. The animal was then given and ate 43 gm. of bread. The respiratory quotients in the subsequent seven consecutive 20-minute periods were as follows: 0.90, .88, .88, .95, .96, 1.00, and .99. These values indicate an immediate and pronounced effect upon the respiratory quotient of this amount of bread. Forty-three grams of bread would contain 26 gm. of dry matter and 98 calories. With excess food the respiratory quotient of the marmot, as with the goose and pig, may be expected to reach an even higher level.

The normal habit of the marmot of eating large amounts of carbohydrate material and transforming it to stored body fat in preparation for the winter sleep was noted in but few of our experiments. Inability to make these animals

⁸ A quotient of about 0.70, but throughout this manuscript this quotient or 0.71 is used as an indication of the fat quotient. It is obvious that rarely, if ever, is there an exclusive fat combustion in hibernation or during fasting. There is invariably a certain proportion of protein oxidized, which may represent not far from 10 to 15 per cent of the total energy production.

eat large amounts ruled out any attempts to try to approximate the stuffing experiments made with the goose and the pig, but in several cases these marmots had eaten enough carbohydrate to yield inside the respiration chamber quotients of 1.00 or above. The highest quotient noted was 1.07 in three successive periods about three hours after the marmot had eaten 108 gm. of laboratory mash.

All these studies accentuate the great desirability, which unfortunately was not realized in our experiments, of having the marmots eat *precisely known amounts of food prior to the experiment*. It was not until fairly late in our research that the laboratory mash (pages 8 and 41) was introduced. Without doubt when the marmot is fed this semi-solid material, a minimum amount of loss would be entailed by the throwing around of food on the part of the animal, and more accurate digestion experiments would result.

Although in most cases our animals showed respiratory quotients at the fat level within 24 hours, a sufficiently large number of instances occurred in which the quotients were not down to this level to argue against the use of the 24-hour fasting period as a preliminary to basal metabolism measurements and the assumption that the fat quotient obtains after that length of fasting. In other words, although in general a 24-hour period of enforced fasting will result in a quotient not far from that of fat, this is by no means always true. With small animals such as rats and birds this may be considered to be invariably the case. A low metabolism results in many instances in a delayed draft upon body material, and in the last analysis the rapidity of the descent of the respiratory quotient depends upon the depletion, first, of the ingested food and, secondly, of the body glycogen deposits. It is, therefore, not always necessary that the respiratory quotient should be 0.71 in order for the heat production to be at the basal level. Hence the first of the two criteria for establishing the basal state need not be considered inviolate. However, the second, that is, whether the heat production descends to a temporarily constant level at any time after food is withheld and at what time this occurs should be carefully noted. (See page 45.)

From the foregoing considerations and from the discussion on pages 45 to 46 it is evident that the length of fasting necessary for establishing a fat quotient may vary considerably, but that it is not essential for the quotient to be at the fat level for basal metabolism measurements. However, the question of the influence of environmental temperature upon the rapidity of the descent of the respiratory quotient needs discussion. A series of experiments were made with marmot A, in all but one instance 24 hours after the last food. The first four experiments were made at 28° C., the usual environmental temperature employed in all our basal studies, and showed respiratory quotients on the four different days of 0.77, .81, .85, and .78. An experiment made at 19° showed 0.74 at only 19 hours after the withdrawal of food. An experiment on November 6, 25 hours after food at a temperature of 14.5°, showed 0.77. These experiments indicate a general trend for

slightly lower respiratory quotients with lower temperatures. They can be explained by the fact that at the rather low temperature the marmot's metabolism was much higher and hence, as the animal was without food, the drafts upon the body glycogen storage were heavier, and consequently the tendency was for the respiratory quotient to be more rapidly depressed.

The hint that the environmental temperature at which metabolism measurements are made has an influence on the respiratory quotient leads to caution with regard to the temperature at which the marmots are living. In our subsequent discussion of the basal heat production (page 52) it will be noted that the zone of thermic neutrality is narrow. This fact accentuates the probable influence of the environmental temperature on the respiratory quotient. It is conceivable that the energy expenditure of a marmot fasting for 24 hours at 16° would be equivalent to that of an animal that had been fasting twice as long, *i.e.*, for 48 hours at 28° C. Hence, if a series of experiments had been made to study the respiratory quotients first after 24 hours and then after 48 hours of fasting, on this principle of reasoning we would expect a more rapid fall from the initial quotient when the animals were kept at 16° than when kept at 28° C. As experiments were not particularly designed to establish this point, the general trend alone can be commented upon. From the entire series of experiments that were made it is clear that there is a somewhat more rapid descent of the respiratory quotient in a given length of time, when the environmental temperature is low.

The drafts upon food and body reserves will likewise be accentuated by activity, and hence a condition where temperature or external stimuli result in activity would likewise hasten the descent of the respiratory quotient. However, this particular factor is so complicating in metabolism experiments that it is avoided under all conditions, if possible. Experiments in which the marmot was vigorously active were usually not completed, or if completed, the calculations of the results were not made because they would be of only incidental value. As in all cases active periods were avoided because distinctly disturbing to basal metabolism measurements and because not infrequently there was a prolonged after-effect of previous muscular activity in periods that otherwise might be assumed to be quiet, the actual experimental evidence as to the descent of the respiratory quotient as a result of activity is not clear. One can readily reason, however, that the activity has precisely the same effect upon drafts on body reserves, especially glycogen reserves, as would the increased metabolism resulting from exposure to severe cold. In respiratory quotient studies obviously both situations should be considered and both severe cold and severe activity avoided.

As an illustration of the extreme changes that can be produced in the respiratory quotient by enforced activity, reference can be made to two experiments. In one case, after a number of periods in which the basal level of the respiratory quotient had been established at 0.74, the chamber was vigorously shaken to produce activity and to stir up the animal. Under these circumstances the respiratory quotient changed in 16 minutes of shak-

ing from a preliminary level of 0.74 to 0.90. In a similar type of experiment with the same animal on the next day, the respiratory quotient changed in 8 minutes from 0.71 to 0.95. After a short time the respiratory quotient returned to the initial fat level, but singularly enough did not go below 0.71.

*The post-absorptive state, as indicated by the level
of the heat production*

In the preceding pages it has been shown that short fasting from 24 to 98 hours is without effect on the rectal temperature of the non-hibernating marmot and that its respiratory quotient usually reaches that of fat at the 48th hour. The effect of the withdrawal of food upon the total heat production was studied at the same time. In the earlier part of the survey, the metabolism was measured immediately after the withdrawal of food and the course of the heat production was studied throughout the first 24 hours of fasting. Marmot X, two hours after eating 56 gm. of bread, had an average heat production of about 590 calories per $10w^{\frac{1}{2}}$ for three periods. At the 20th hour it had fallen to about 400 calories. From the 21st hour on the heat production varied at not far from 300 calories. This was absolutely the lowest value obtained with marmot X and, as can be seen from table 6 (page 61), this particular minimum value of about 300 calories was actually lower than the average basal value ascribed to this animal of 370 calories, which includes the average of two other days. Thus a plateau was rapidly reached so far as the heat production was concerned. Meanwhile at no time during this experiment of January 3rd had the respiratory quotient gone below 0.83. Indeed, 22 hours after the withdrawal of food the quotient was 0.92.

From the experiments already referred to (page 42) as having contributed to the story of the respiratory quotient on the first four days of fasting, we likewise have data with regard to the heat production. Here again it is clear that after 24 hours the heat production was practically unaltered by a longer fast. In general, the heat production was essentially at its lowest point or at least without significant decrease after 24 hours of fasting. This, therefore, confirms our belief that, so far as the basal metabolism of the non-hibernating marmot is concerned, withdrawal of food for 24 hours is sufficient.

This length of fast, however, does not always result in a fat quotient. Indeed, in one instance a quotient in the neighborhood of 0.90 was noted. Under ordinary conditions with other animals this would be interpreted as meaning a lag in the after-effect of food, with probably an increased metabolism due to the stimulus of food, and hence this animal would hardly be considered as being at the basal metabolic level.

In table 5 (pages 57 to 59) have been summarized the metabolism measurements made at 28° C. on non-hibernating marmots, fasting from 24 to 48 hours. The italicized values represent the minimum basal values selected for averaging. High respiratory quotients appeared in these selections frequently, although not accompanied by high heat production. Indeed, more

often than not these high respiratory quotients were accompanied by extremely low, if not the lowest, heat production. This was found so frequently that it challenged the method of determination, particularly of the oxygen consumption. If there was a systematic error in the determination of the oxygen whereby the oxygen measurements were too low, this would ultimately result in a high respiratory quotient and likewise, as the heat production was calculated from the oxygen consumption, in a low heat production. However, in a number of these instances where there was a high respiratory quotient accompanied by a relatively low heat production, the analyses were all made on the Carpenter gas-analysis apparatus by an extremely accurate technician, Mr. George Lee, and were checked by one, if not two, other analysts. Hence it is believed that this phenomenon is not due to defective oxygen determinations. Consequently the after-effect of the previous ingestion of an excessive amount of carbohydrate, which results in a persistently high respiratory quotient, is without any significance upon the heat production.

Muscular repose

In all basal metabolism experiments precaution is taken to rule out body activity. With some animals, as the mouse, this is almost impossible. With the large ruminant the movements can be confined, for the most part, to ordinary stall activity which, according to Ritzman,⁹ results in an increase in metabolism above the basal or standard level of not much more than 15 per cent. With the marmot usually we were not interested in periods of activity. They were of no value, and consequently in relatively few active periods were metabolism measurements made. In common with the techniques used for all other animals, we felt that graphic records of the degree of repose of our marmots were indispensable. Hence in nearly every experiment graphic records of the degree of repose or of activity were secured and those periods with significant activity as indicated by these records were disregarded and frequently not even calculated. In those cases where the calculations were actually made, the increases in heat production, although not invariably closely associated with the intensity of movement as shown by the graphic records, did roughly parallel the activity records.

Special interest attaches to two experiments at 28° in which enforced activity was induced by shaking the marmot's cage. These have already been cited (page 44) in the discussion of the effect of this activity on the respiratory quotient. In both cases the marmot had been essentially 30 hours without food. Marmot O was studied on May 4, 1935, in five consecutive periods without any shaking of its cage, giving a minimum metabolism of 602 and a maximum metabolism of 670 calories. On May 14 with considerably more voluntary activity than recorded by the kymograph record of May 4, the animal showed in six periods the following rates of heat pro-

⁹ Ritzman, E. G., and F. G. Benedict, Carnegie Inst. Wash. Pub. No. 494, 1938, p. 99.

duction: 906, 1036, 1153, 1259, 988, and 969 calories per $10w^{\frac{1}{2}}$. After the last period the small respiration chamber in which the animal was confined was shaken vigorously by the observer, and the metabolism was sustained at a rate of 2771 calories for the 16 minutes of shaking. Compared with the minimum value as found on May 4 of 602 calories, there was nearly a 5-fold increase in heat production as a result of the enforced activity. The same marmot was studied the next day, 53 hours after food, and in the first three periods had a heat production of 712, 765, and 708 calories. The chamber was then vigorously shaken for 18 minutes, and the metabolism was sustained at the incredible rate of 4458 calories for the last 15 minutes of shaking. This shows a 7-fold increase above the actual minimum recorded for this animal.

A hint as to the actual amount to which the animal could raise its heat production by its own activity inside the chamber was obtained in two experiments on No. 5 on September 29 and October 1, 1936. On September 29 four well-agreeing 20-minute periods gave 555 calories. A subsequent period gave 1337 calories, or a doubling of the metabolism. Essentially the same picture was shown on October 1 with, however, the initial metabolism being more nearly 400 calories, and in this case the metabolism rose in one period to 1387 calories. No. 10 on September 28 had a minimum heat production of 544 calories, which it increased by activity to 1180 calories, thus doubling its metabolism. These experiments afford evidence that the marmot might double its metabolism by voluntary activity, and that enforced activity (shaking the animal) may increase its metabolism 6- or 7-fold.

With the marmot, as with other animals, it is desirable to measure the true basal metabolism with as small a probable error as possible. If an animal can by its own activity increase its metabolism 100 per cent, obviously to secure basal values within ± 15 per cent of the true level requires the greatest care to insure that activity is ruled out and to insure that suitable graphic records of the degree of repose accompany each experiment. The extreme metabolic reaction (100 per cent increase) of the marmot as a result of voluntary activity inside the respiration chamber means that one must give more careful consideration to the otherwise seemingly rather minor activities that might influence its metabolism. These activities may be considered in the case of this wild, ferocious animal under two heads, (1) the emotional element, apprehensiveness, or anxiety, and (2) the true body movements. The body movements have been studied, with the use of kymograph records as the major index of activity, and their profound influence on the metabolism is too well known to require further discussion. The minor activities, which might almost be classed as emotional, may be ascribed to three causes: (1) the adjustment of the animal to laboratory conditions, a strange environment, and handling by the observer prior to the experiments, an adjustment that means in large part the matter of overcoming fright, (2) the noise in the laboratory, which may affect the marmot during a period of basal metabolism measurement, and (3) the possibility of diurnal functions which may be influ-

enced not simply by the time of day but likewise by the inevitably lessened noise during an evening series of measurements.

Laboratory adjustment. To note whether the metabolism is influenced by adaptation to laboratory conditions, a few marmots were studied shortly after they reached the Laboratory as well as subsequently. Therefore, when first studied these animals were nearer their native-life phase than many of the others. With two of these animals the highest metabolism values found in the entire series were noted at this time. With three others there was no significant change in metabolism, as the first measurement was as low as, if not lower than, the others. It would thus appear that many of these marmots rather rapidly adjust themselves to laboratory conditions and, furthermore, that the violence incurred in adjusting them to laboratory food and environment was not sufficiently great to alter the metabolic level.

Noise. The reaction of various animals to noises has been frequently noted in the laboratory. The marmots were measured always in complete darkness, but in metallic chambers not immersed in water and hence rather easily conducting sound. Special experiments were, therefore, directed toward increasing the noise rather than decreasing it. On two occasions with marmot B we placed a bell inside the chamber and rang it loudly several times throughout the periods of measurement. Unfortunately these experiments were made at a chamber temperature of about 10°, under which condition there was normally a high metabolism. The kymograph records showed that the animal engaged in considerable activity. As a result of fifteen consecutive 45-minute periods, during which the bell rang continuously in the first six periods, no evidence of an effect upon metabolism due specifically to the bell could be found. The metabolism throughout the entire time was essentially 1200 calories per $10w^{\frac{2}{3}}$ with an animal weighing 3.01 kg. This experiment was made 27 hours after food, and the respiratory quotient was 0.71. The rectal temperature at the beginning was 38.4° and at the end 37.8° C. In the second experiment with marmot B on June 10, when it weighed 3.15 kg. and had been 26 hours without food, the environmental temperature in sixteen consecutive 45-minute periods rose from 9.3° to 15.7° C. The bell was ringing in all periods except period 7. The heat production in the successive periods was as follows: 932, 1027, 1033, 984, 943, 909, 755, 857, 1153, 1234, 1151, 741, 566, 691, 794, 876. In the seventh period, the only one in which the bell was not ringing, there was a momentary decrease in the heat production to 755 calories, immediately followed, however, by a continued rise, but the effect of habituation to the noise is shown definitely toward the end of the experiment when the heat production approximated, on the average, not far from 700 calories per $10w^{\frac{2}{3}}$ for the last five periods. This metabolic level was also closely associated with a much higher environmental temperature of 15.3° C. The results therefore reflect the effect of the continually rising temperature and the noise. In both the experiments no significant effect of the noise *per se* can be stated to have been found.

Influence of time of day. With a few marmots tests were made to study this point. The first series was made with marmot B in 1932 by our late colleague, Mr. E. L. Fox. The environmental temperature was in this early experiment 24° rather than 28°, and the heat production was 643 calories in the day periods and 533 calories in the night periods. On another day, June 28, the animal was measured at 22°, and the day experiment showed 558 calories and the night 601 calories. Thus we have a reversed situation here, for in one instance the night shows a lower and in the other instance a higher metabolism. A special study was made on June 22 with this same animal at a low temperature. The animal had been at an environmental temperature of 24° and was then placed in an iced respiration chamber, which had a temperature of 14° C. The day experiment gave 794 calories and the night experiment 791 calories, thus indicating no difference in metabolism. In November, 1936, Nos. 3 and 4 were studied at an environmental temperature of 28°. The previous environmental temperature had been between 27.5° and 30°, and they had been kept in a dark room. The metabolism of No. 3 was 387 calories at 2.13 p.m., 438 calories at 5.35 p.m., and was fairly constant throughout the evening at about 430 calories. The metabolism in the evening was slightly higher than that in the afternoon. With No. 4 the situation was slightly reversed, the metabolism in the period in the afternoon being highest, 315 calories, and in the next three periods being somewhat under 300 calories. The extraordinarily low values noted with No. 4, that is, values averaging a little under 300 calories per $10w^{\frac{3}{4}}$, will be discussed later (page 62) in considering the basal metabolism. The main deduction is that the time of day is entirely without effect upon the metabolism of the marmot. It has been found with many animals and particularly with birds that the diurnal rhythm persists and that, although measured in complete darkness, many show a definitely lower metabolism during the night. It is obvious that during the night and evening hours there are less extraneous noises around the Laboratory and on the grounds than during the day. However, the Laboratory is located away from a main thoroughfare, and there is not an unusual amount of traffic and undue noise about the building. Hence although with many animal species it has been found that there is a true influence of the time of day, this does not obtain in the case of the marmot.

Zone of thermic neutrality

All the precautions that experimenters have found necessary to be taken in establishing the basal metabolism of any animal, such as repose and the post-absorptive condition, obviously must be considered with the marmot. In addition, owing to its well-known semi-poikilothermic nature, special attention must be given to the consideration of the limits of the zone of thermic neutrality. With an animal having a fluctuating body temperature even in the non-hibernating condition, the question as to just at what point the animal begins to produce heat to combat cold, on the one hand, or at

what point the temperature regulation is so defective that there is a hyperthermy, on the other hand, needs special study. With most laboratory animals, aside from the mouse and the rat, the zone of thermic neutrality can be fairly easily established and is reasonably wide. As we have already seen, the rectal temperature of the marmot is dependent to a great extent upon the environmental temperature. The question may be fairly asked as to what is the normal rectal temperature of the non-hibernating marmot. It has been stated on page 34 that when the marmot is kept at 16° C. the rectal temperature is about 36° C., when it is kept at about 28°, its body temperature is about 37° C., and when kept at 34° it is at still another level, approximately 39° C. The decision as to which of these temperatures represents the normal rectal temperature is closely tied up with the establishment of the metabolic level. That rectal temperature at which the non-hibernating marmot has its minimum metabolic activity may be considered to be more nearly the normal temperature than is a rectal temperature when the animal is producing heat to combat cold. The influence of the *environmental temperature* upon the metabolism is another story. Based upon observations on other animals one could predict without any measurements whatsoever upon the marmot that a low environmental temperature would ultimately produce a higher metabolism in this animal. The question is, how low a temperature? In its comparative physiological study of heat production, the Nutrition Laboratory has measured the metabolism of its different species of animals not only at thermic neutrality but likewise as nearly as possible at the "laboratory temperature" used by Rubner in his study of the basal metabolism of different animals, that is, at or very near to 16° C. In so doing, the Nutrition Laboratory recognized that 16° may be considerably below the lower limit of the zone of thermic neutrality for some animals and well within the zone for others. With the marmot, therefore, our study takes two parts, (1) the attempt to establish as closely as possible the zone of thermic neutrality and (2) the measurement of the metabolism definitely at 16° C. Since the concept of Rubner, another factor has been introduced into the study of the metabolism of an animal, wholly unsuspected at that time, that is, the adaptability of an animal to a given environmental temperature. Nowhere has this point been more carefully studied and elucidated than in the laboratory of Giaja and his colleague Gelineo at Belgrade.¹⁰ Hence the influence upon metabolism of prolonged sojourns at various temperatures and the rapidity of adaptation at these temperatures become essential parts of the study of the marmot.

The first phase of our study of environmental temperature and one of immediate interest is the establishment as definitely as possible of the zone of thermic neutrality. The second phase is to note the effect of a prolonged stay at a certain environmental temperature upon the course of the metab-

¹⁰ Gelineo, S., Annal. de Physiol. et de Physicochim. biol., 1934, **10**, p. 1096; Giaja, J., Biologie Médicale, 1935, **25**, p. 209; Giaja, J., and S. Gelineo, Annal. de Physiol. et de Physicochim. biol., 1931, **7**, p. 163.

olism of the animal at that temperature, in other words, its adaptation. The third is a specific study with the animal exposed to 16°, with emphasis upon the rapid transition from higher temperatures to 16° and then, conversely, the rapid transition from 16° to 28° C. For this type of investigation it is obvious that a series of experiments is necessary, for a few records could be of little, if any, help. Much of our earlier work on the marmot at the Nutrition Laboratory was carried out on the assumption that a temperature of 28° was for most animals within the zone of thermic neutrality, and no attempts were made at that time to outline or define this zone sharply. Later the effects of slight variants from this temperature were noted, and an attempt was made to outline the zone more sharply. With an animal like the marmot, subjected to great differences in environmental temperature in its wild life, one might expect the zone of thermic neutrality to be wide. The heavy fur of the marmot would likewise support this conception. The large domestic animals, the sheep especially which is well protected by its fleece, have a wide zone.

Starting out on the assumption that 28° C. or thereabouts would be well within the zone of thermic neutrality for the non-hibernating marmot, we made one of our chief studies for sharply demarking the zone with animal R. In October 1935, during which time the weight changes were pronounced, the heat production of this marmot measured on several days at 28°, usually fasting 24 hours, was 429 calories on October 3 and 483 calories per 10w³ on October 9. On October 10 it averaged about 400 calories and on October 14, 533 calories. On October 18 the temperature was lowered to 26°, and the average heat production was 672 calories. On October 21 at 24.5° it was 814 calories; on October 25 at 22°, 800 calories; on October 28 at 20°, 955 calories; and on November 1 at 18°, 965 calories. Then, as a control, the marmot was studied again on November 12 at 27.5°, when the heat production was 380 calories. From this study it would appear clear that at 28° the metabolism is at the minimum level and that it increases rather rapidly with a lowering of the temperature amounting only to two or three degrees. The lower limit of the zone of thermic neutrality for marmot R would therefore be considered to be not far from 27.5° C.

On December 5 with marmot U an experiment was made to establish the upper limit of this zone. At 28° the animal showed on October 12, 17, and 26 values of 531, 529, and 584 calories, respectively. On all these dates it had been 24 hours without food. In this particular case, however, we were dealing with a small, probably immature animal weighing but a little over 1 kg. On December 5 animal U had been kept at 28° for some time and was studied at temperatures of about 30° for the first two periods, when the metabolic rate was 555 calories. In the next three periods at 31° it was 627 calories, and in the next three periods at 34°, 703 calories. These results show clearly that with this animal temperatures above 30° were provocative of measurably increased heat production.

With marmot Y a similar experiment was made on December 5 at sub-

stantially the same temperatures as with animal U. The animal, which had been maintained for some time beforehand at 28° and was measured at 28.5°, had a heat production of 422 calories. At 32° for three periods the heat production was 488 calories and at 34.5° it was 590 calories. At the end of this time the rectal temperature of the marmot was abnormally high, 40.3° C.

A year later No. 7, weighing 3.4 kg., under the standard conditions of 24 hours fasting, and measured and kept previously at 28°, showed repeatedly a metabolism of essentially 400 calories. On November 12 the average of four periods at 28° gave 420 calories. The temperature of the chamber was then lowered to 25°, and the average of four periods gave 540 calories, showing again the extraordinarily close line of demarkation of the lower limit of the zone of thermic neutrality. It is, therefore, clear that our first series of experiments made at 28° were at a temperature that was fortunately as near as possible to what one might term the critical temperature. Indeed, the marmot has, as have very few animals, such a narrow zone of thermic neutrality as to confirm one point, *i.e.*, that 28° may be taken as the critical temperature and that temperatures a degree or two above or below this point are outside the very narrow zone of thermic neutrality. It is thus obvious that for all comparative purposes and apparently (as it seems from the wide difference in the sizes of the marmots used in this study) irrespective of size, metabolism experiments on the non-hibernating marmot made at 28° alone may be used, for only at 28° C. will the metabolism be unaffected by environmental temperature.

In this study a distinct tendency for adaptation was noted. A study of the adaptation especially at the higher temperatures was, therefore, made. In view of the extremely low basal metabolism of the marmot in the zone of thermic neutrality, in general not far from 400 calories per $10w^{\frac{3}{4}}$, *i.e.*, a metabolism much lower than that of all other warm-blooded animals certainly of this size, the question naturally arose as to whether or no any conditions could be established where the metabolism would be even lower than this already very low level. For this purpose several of the marmots were exposed for long periods to temperatures of 32° or thereabouts, a temperature already established as above the critical temperature, and then their metabolism was measured after these various periods of exposure to note if there was any evidence of adaptation. The most extended series was that made with marmot S in the fall and winter of 1935–36. This animal, weighing 3 kg. or over, had a metabolism when measured at 28° on October 2, 11, 22, and 29, and December 4 and 18 as follows: 378, 328, 371, 447, 473, and 432 calories. It was then exposed for 7 weeks to 32° to 36°, at the end of which time, that is, on March 2, 1936, its heat production was 497 calories. On March 6 it was 479 calories. It is thus seen that the reaction to a warmer environmental temperature which was so pronouncedly shown by marmot Y was here practically absent. Animal S had adjusted itself to the higher temperatures. Its rectal temperature at the end of the experiment on March 2 was 39.4° and on March 6 it was 38.9°, which is definitely

higher than the average body temperature at 28° C. Thus it appears that marmot S underwent a marvelous metabolic adaptation to these unusual conditions, accompanied by an abnormally high rectal temperature, and yet the actual heat production, although higher than in some of the earlier experiments at 28°, was not significantly higher than the average for many of the marmots at the critical temperature of 28°.

A second series of experiments on Nos. 23, 24, 26, and 27 throws some light upon the question of adaptation in a different manner, inasmuch as these marmots had been kept in a garage for at least 48 hours at 8° C. and were then brought into the Laboratory and studied at 28° C. Their heat production, measured in six 20-minute periods, averaged 415, 403, 410, and 374 calories, respectively. (See table 5, page 59.) These values average not far from 400 calories. This series of experiments speaks for a rapid adjustment to the more favorable temperature of 28° C. Consequently, although we have in most of our studies maintained our marmots at the environmental temperature of 28° previous to basal measurements, we feel that this may not be necessary. The Belgrade investigators, Giaja and Gelineo, have seriously recommended that rats, for example, must be kept for two weeks at the temperature at which they are to be measured, during which time there is a continued period of adaptation. Here the evidence is that, although the marmot has a narrow zone of thermic neutrality, almost a single temperature, nevertheless the period of adaptation is fairly rapid and no accuracy is sacrificed by not maintaining this animal for two or three weeks at the optimum temperature prior to measurement.

RELATIONSHIP BETWEEN RECTAL TEMPERATURE AND METABOLISM

Assuming for the moment that the rectal temperatures of all the marmots were obtained under a uniform condition so as to result in the absence of extreme activity at the time of measurement and that they were measured following the metabolism measurement and thus this procedure did not affect the metabolism, we may examine the data in table 5 (pages 57 to 59) and note with the same animal how the rectal temperature is affected or changed by the metabolism. With marmot A the highest rectal temperature is associated with the lowest metabolism. With B there is a great difference in the two values for metabolism with an insignificant alteration in body temperature. With H the maximum temperature is associated with the lowest metabolism. With I practically all the temperatures are high and the metabolism is high. With K the lowest rectal temperature is associated with the lowest heat production. With S again the lowest temperatures are associated with the lowest heat production. With U on the days when the temperature was measured, the lowest heat production is with the lowest rectal temperature. With V in two experiments showing a difference of 3° in rectal temperature, there is no change in the heat production. With X a change of 3.3° in the extremes in rectal temperature is accompanied by a very great difference in heat production, the lowest rectal temperature occur-

ring with the lowest heat production. With No. 1 the highest rectal temperature is noted with the highest heat production. With No. 2 the highest rectal temperature occurs with nearly the highest heat production, but on days when the rectal temperature was 37.8° almost the greatest difference in heat production is found. No. 3 is characterized on one day by a high rectal temperature of 39.0° and a high heat production, but essentially the same heat production was noted on a later day when the temperature was practically three degrees lower. With No. 4 the lowest rectal temperature was 34.0°, and this was accompanied by the lowest heat production, but on the next experimental day the temperature was 2.4° higher and the heat production was not significantly altered. With No. 5 the highest rectal temperature, 39.2°, accompanies nearly the lowest heat production, 385 calories. With No. 6 the highest rectal temperature, 37.7°, occurs with the highest heat production. With No. 7 the same picture is shown as with No. 6. No. 8 had a high metabolism on the last day, with the highest rectal temperature, 39.0° C. When its rectal temperature was 38.9° the heat production was 476 calories, almost the lowest with this animal. With No. 9 rectal temperatures of 38.2° or over were found on both the days with about the highest and about the lowest heat production. With No. 10 a high average body temperature prevailed. The lowest heat production was found with a rectal temperature of 38.3° C. The last five animals listed in table 5, each studied on one day only, had for the most part fairly low temperatures, probably explained by their long previous sojourn at 8° C., and low metabolisms.

This inspection of the individual values for the same marmot from day to day shows that, in spite of the irregularity in the temperatures and the difficulty in determining them, there is a trend towards a somewhat higher metabolism with a higher rectal temperature. But it is hardly more than a trend and is far from being sufficiently well established to justify the statement that the rectal temperature is of itself a controlling factor in the heat production. This conclusion removes the marmot completely, at least in the non-hibernating stage, from the class of poikilothermic animals whose rectal temperature is often considered in large part the determining factor in metabolism or is closely associated with the metabolic level. From this standpoint, therefore, the non-hibernating marmot may be considered a warm-blooded animal. Probably the absence of correlation between heat production and rectal temperature may be more marked with the marmot than with many other animals including man, who shows very pronouncedly an influence of rectal temperature upon the basal metabolism. In fever, for example, a relatively slight rise in the body temperature of the human is accompanied by a highly elevated heat production.

This entire discussion thus far has been based upon the comparison of the rectal temperatures and the metabolisms of one and the same marmot on different days and leads to the conclusion that there is little, if any, correlation between the temperature of the animal and its heat production. As

the rectal temperature studies show that there are certain animals that seem usually to have relatively high temperatures and others relatively low temperatures, a comparison of the average rectal temperatures and the average heat productions of the various animals is justifiable. The average rectal temperature of each marmot, noted on days when its metabolism was measured, is recorded in table 6 (page 61). The minimum temperature is 34.3° C. with marmot R, based unfortunately upon only one experiment, although the metabolism was measured on three days. The maximum is 38.4° with marmot I, based upon five days. There are, however, three other animals, H, 8, and 10, that have values 38° or above, that for H based upon the average of two days, that for No. 8 based upon the average of 7 days, and that for No. 10 based upon the average of 11 days. At the lower temperature level, there are four marmots other than R with a rectal temperature 36° or below. The average minimum heat production per $10w^{\frac{1}{2}}$ of each marmot is also recorded in table 6. Inspection of the values for those animals with relatively high rectal temperatures shows that there is a distinct tendency for those with temperatures of 38° and over to have a heat production of 460 calories or over (460, 470, 460, and 470 calories). On the average the rectal temperatures of those marmots having a heat production of 460 calories or over (animals B, H, I, V, W, 8, and 10) is 37.6° C. Conversely, the average rectal temperature of those marmots (S, U, X, Y, 1, 4, 6, and 27) having a heat production of 370 calories or below is 36.4° C.

From this crude inspection there would appear to be a slight correlation between the rectal temperature and the heat production. This apparent slight correlation between rectal temperature and heat production shown by comparing different marmots is at variance with the first conclusion, *i.e.*, that with one and the same marmot there is no correlation between the rectal temperature and the heat production. As the greatest importance of the rectal temperature study in connection with the study of the basal heat production is to note whether with the *individual* marmot alone the metabolism can be considered to be basal when accompanied by an average rectal temperature (*i.e.*, not high or not low), greater stress must be placed on the conclusion derived from the first comparison, namely, that there is an *apparent absence of any correlation* between basal metabolism and rectal temperature.

It is hardly likely that these marmots were of different races, although the fact that there are certain groups that show consistently a high rectal temperature and other groups that show consistently a low rectal temperature might suggest that such a racial difference existed. From the standpoint of studying the basal metabolism, however, the conclusion may be drawn that, other things being equal, it is not justifiable to insist that the rectal temperature of the marmot should be at 37° C. (the average level at an environment of 28° C.) prior to making basal metabolism measurements. The fact that the labile body temperature (34° to 39° C.) of the non-hiber-

nating marmot does not affect significantly its basal metabolism is in contrast to the noticeable changes in the heat production of other animals (notably mice and rats) accompanying the same range in body temperature.

BASAL METABOLISM

For basal metabolism measurements on the marmot, as demonstrated in the preceding pages, it is necessary to avoid such conditions as would affect the metabolism, namely, too low an environmental temperature, too short a period of fasting, muscular activity, and any semblance of hibernation. A large number of experiments made under the prerequisites for basal measurements were carried out on 28 marmots, covering a period from September 1931 to and including February 1937. For each individual marmot we have presented, in table 5, the data for the body weight, the rectal temperature, the respiratory quotient, and the heat production on the three bases of expression, total, per kilogram of body weight, and per $10w^{\frac{1}{2}}$. The rectal temperature was usually taken at the end of the experiment. In general the practice of taking the temperature before the experiment is considered inadvisable, as it agitates the animal and thereby increases its metabolism. As a rule the quotients on any individual day were reasonably uniform. The variations in the quotient have already been discussed. The main object of presenting the data in table 5 is to consider the variability in the heat production. Usually the body weight of each marmot throughout the experimental season, which in most cases covered a relatively short period of not more than two months, did not undergo any significant variations. In a few instances, which will be cited later, there were some changes in body weight which should be taken into consideration in the interpretation of the results.

The first animal showing pronounced variation is marmot I, with a heat production ranging from 664 calories on December 14 to 436 calories on January 16. With marmot U the heat production ranges from 584 calories on October 26 to as low as 343 calories on December 18. With No. 1 the range is from 511 calories on October 20 to the extremely low value of 292 calories on November 4 and with No. 2 from 529 calories on September 29 to 373 calories on November 10. With No. 4 the highest value recorded is 466 calories on September 18 and the two values a little below 300 calories on November 4 and 19 were the lowest. With No. 9 the maximum is 604 calories on September 25 and the minimum is 425 calories on November 4. In the series with No. 10 the variations are much less, only from 609 calories on October 6 to a minimum of 465 calories on September 21. The last four marmots, upon which only one determination each was made, had been kept in a cold garage at 8° for at least 48 hours and then were measured at 28° C. These marmots all show values close to 400 calories, although in only one experiment and obviously the first experiment with these animals. When the values for the different marmots are compared, the range in the entire series of experiments is from a maximum of 664 calories with marmot I on December 14 to a minimum of 276 calories with No. 4 on November 4.

TABLE 5—*Metabolism of non-hibernating marmots*
(Fasting 24 to 48 hours; measured at 28° C.)

(Values in italics are considered to represent the minimum basal metabolism)

Marmot	Date	Body weight	Rectal temperature	Respiratory quotient	Heat production per 24 hours		
					Total	Per kg.	Per 10w ²
A	Sept. 23, 1931	1.4581	57.2	39.6	447
	Nov. 12, "	1.53	38.1	.85	56.2	36.7	423
	" 18, "	1.64	37.3	.78	63.8	41.5	491
B	Nov. 10, 1931	2.15	37.1	.76	88.5	41.1	530
	" 17, "	2.21	37.3	.78	78.8	35.7	463
H	Dec. 5, 1933	2.69	39.8	.76	96.4	35.8	447
	" 19, "	2.58	36.7	.80	91.3	35.4	485
	Jan. 9, 1934	2.8981	97.7	33.8	481
I	Dec. 7, 1933	2.50	41.5	.79	120.3	48.2	654
	" 14, "	2.41	38.5	.76	119.5	49.6	664
	" 21, "	2.30	36.8	.76	84.0	36.5	483
	" 28, "	2.38	38.8	.73	100.4	42.2	564
	Jan. 10, 1934	2.38	36.3	.71	84.6	35.6	476
	" 16, "	2.4771	79.9	32.4	436
K	Dec. 16, 1933	1.62	35.8	.75	58.3	33.9	422
	" 23, "	1.56	37.5	.70	70.9	52.9	529
	Jan. 4, 1934	1.62	36.7	.72	64.0	45.4	464
M	April 11, 1934	2.5076	89.1	35.6	484
	" 15, "	2.4881	95.9	38.7	524
	" 22, "	2.4173	98.2	40.8	546
	" 25, "	2.5971	89.3	34.5	474
	" 30, "	2.6171	100.5	38.6	531
R	Oct. 10, 1935	2.8074	78.5	28.0	396
	Nov. 12, "	2.41	34.3	.73	68.4	28.4	380
	Dec. 16, "	2.8981	97.3	33.7	480
S	Oct. 2, 1935	3.12	37.7	.75	80.6	25.8	378
	" 11, "	2.91	35.3	.72	67.0	23.0	328
	" 22, "	2.91	35.3	.76	76.8	25.8	371
	" 29, "	2.9973	92.8	31.0	447
	Dec. 4, "	3.43	37.9	.69	107.5	31.3	473
	" 18, "	3.3674	96.8	28.8	432
U	Oct. 12, 1935	1.04	37.4	.71	54.5	52.4	531
	" 17, "	1.0477	54.3	52.3	529
	" 26, "	1.08	37.7	.80	61.5	56.9	584
	Dec. 9, "	1.76	35.9	.78	59.6	33.9	409
	" 18, "	1.7385	47.1	28.6	343
	" 24, "	1.7183	51.0	29.8	357
	" 30, "	1.7676	60.3	34.3	414
V	Oct. 15, 1935	1.87	35.0	.79	86.0	46.0	567
	" 23, "	2.04	38.1	.79	90.0	44.1	560
W	Oct. 16, 1935	1.15	36.2	.76	54.6	47.4	477
	" 24, "	1.32	36.9	.87	72.2	54.7	600
	Dec. 16, "	1.8681	66.7	35.9	441
X	Oct. 30, 1935	3.18	37.5	.76	101.4	31.6	468
	Dec. 9, "	3.40	37.1	.78	102.6	30.2	455
	" 17, "	3.5189	93.6	26.7	405
	" 24, "	3.47	35.3	.73	96.0	27.7	419
	Jan. 3, 1936	3.55	34.2	.87	69.0	19.4	296

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TABLE 5—Continued

Marmot	Date	Body weight	Rectal temperature	Respiratory quotient	Heat production per 24 hours		
					Total	Per kg.	Per 10w ^{1/2}
Y	Oct. 31, 1935	1.93	37.1	.81	89.7	46.5	579
	Dec. 5, "	2.4270	76.7	31.7	425
	" 9, "	2.47	36.0	.72	79.1	32.0	433
	" 17, "	2.4269	63.1	26.1	350
	" 23, "	2.3869	56.1	24.4	320
	" 30, "	2.3679	60.8	25.7	340
1	Sept. 17, 1936	3.37	36.4	.73	103.4	30.5	460
	" 21, "	3.45	37.0	.73	100.7	30.4	453
	" 24, "	3.30	35.1	.73	70.9	21.5	320
	" 28, "	3.27	35.1	.72	80.2	24.5	364
	Oct. 20, "	3.24	38.1	.80	114.3	34.2	511
	" 21, "	3.24	36.5	.70	88.0	27.2	402
	" 26, "	3.21	36.3	.74	72.1	22.5	331
	Nov. 4, "	3.18	37.5	.68	63.2	19.9	292
	Sept. 17, 1936	2.90	38.4	.73	102.7	35.4	505
	" 22, "	2.48	37.8	.72	95.8	38.6	523
2	" 25, "	2.54	36.3	.75	89.0	35.0	479
	" 29, "	2.51	37.8	.69	97.6	38.9	529
	Oct. 13, "	2.60	37.8	.84	73.4	28.2	388
	" 14, "	2.55	36.3	.75	74.1	29.1	397
	Nov. 9, "	2.66	36.9	.78	77.5	29.1	404
	" 10, "	2.58	36.5	.73	70.3	27.4	373
	Sept. 15, 1936	2.37	36.6	.78	98.6	40.8	555
	" 18, "	2.48	39.0	.79	102.6	41.4	560
	" 22, "	3.04	36.5	.73	98.6	32.4	470
	" 28, "	3.12	37.5	.74	91.8	29.4	430
3	Oct. 6, "	3.1470	115.2	36.7	538
	" 13, "	3.16	36.1	.77	120.9	38.3	562
	" 14, "	3.10	36.3	.70	82.2	26.5	386
	Nov. 9, "	3.27	36.1	.70	103.2	31.6	469
	" 10, "	3.22	36.7	.72	92.8	28.8	426
	" 19, "	3.08	35.7	.70	80.0	26.0	378
	Sept. 18, 1936	2.75	36.4	.73	91.5	32.9	466
	" 24, "	2.70	35.4	.73	81.1	30.0	419
	" 30, "	2.73	36.9	.74	89.2	32.7	457
	Oct. 20, "	2.97	37.2	.87	77.2	26.0	373
4	" 21, "	2.92	36.0	.73	68.7	23.5	337
	" 26, "	2.93	36.1	.91	66.3	22.6	323
	" 27, "	2.87	35.7	.72	62.3	21.7	308
	Nov. 4, "	2.78	34.0	.70	54.6	19.6	276
	" 19, "	2.68	36.4	.69	55.0	20.5	285
	Sept. 16, 1936	2.68	37.9	.72	90.3	33.7	468
	" 23, "	2.68	36.3	.75	102.5	38.2	531
	" 25, "	2.71	36.8	.87	73.6	27.2	379
	" 29, "	2.6273	105.6	40.3	555
	Oct. 1, "	2.71	39.2	.84	74.9	27.6	385
5	" 2, "	2.57	37.0	.76	87.7	34.1	467
	" 8, "	2.45	38.3	.81	102.0	41.6	561
	" 9, "	2.3873	72.8	30.6	408
	Oct. 2, 1936	2.1676	80.9	37.4	484
	" 5, "	2.22	37.7	.74	100.9	44.1	593
	" 8, "	2.22	36.5	.78	62.6	28.2	368
6	" 9, "	2.14	36.2	.72	69.1	32.3	416
	" 27, "	2.32	36.2	.75	82.2	35.4	471
	" 28, "	2.26	36.2	.73	64.5	28.5	374

TABLE 5—*Concluded*

Marmot	Date	Body weight kg.	Rectal temperature °C.	Respiratory quotient	Heat production per 24 hours		
					Total cal.	Per kg. cal.	Per 10w cal.
7	Oct. 2, 1936	3.30	38.0	.71	125.7	38.1	567
	" 7, "	3.20	37.7	.70	105.6	33.0	486
	" 27, "	3.51	35.8	.87	86.9	24.8	376
	" 28, "	3.41	36.7	.78	92.7	27.2	410
	Nov. 11, "	3.46	37.4	.77	94.6	27.3	418
8	Sept. 16, 1936	2.21	37.7	.73	76.4	34.6	450
	" 19, "	2.18	38.5	.72	79.6	36.5	474
	" 23, "	2.08	37.3	.70	74.9	36.0	460
	" 28, "	2.06	38.9	.73	77.1	37.4	476
	Oct. 13, "	1.90	37.3	.76	74.2	39.0	484
	" 14, "	1.89	37.1	.71	69.8	36.9	457
	Nov. 5, "	1.98	39.0	.74	93.4	47.2	593
9	Sept. 21, 1936	3.32	37.8	.69	111.9	33.7	503
	" 23, "	3.39	38.3	.72	118.2	34.9	523
	" 25, "	3.41	37.8	.74	136.9	40.2	604
	" 30, "	3.35	38.3	.74	131.3	39.2	587
	Oct. 8, "	3.34	36.9	.75	130.6	39.1	552
	" 27, "	3.51	38.3	.79	132.5	37.7	573
	" 28, "	3.42	37.5	.72	110.9	32.4	489
	Nov. 4, "	3.34	36.0	.69	94.9	28.4	425
	Jan. 15, 1937	3.74	38.1	.79	139.4	37.3	579
	" 16, "	3.58	38.2	.68	104.3	29.1	446
	Feb. 10, "	3.52	38.2	.72	124.8	35.4	539
	Sept. 21, 1936	4.80	38.3	.72	132.5	27.6	465
	" 24, "	4.78	38.5	.71	153.5	32.1	541
	" 28, "	4.75	37.8	.70	153.8	32.4	544
10	Oct. 1, "	4.67	39.1	.72	139.5	29.9	499
	" 5, "	4.65	37.9	.80	130.3	28.0	568
	" 6, "	4.59	38.9	.72	168.2	36.6	609
	" 13, "	4.40	37.8	.82	140.3	31.9	522
	" 14, "	4.35	37.4	.74	124.7	28.7	468
	Nov. 5, "	3.90	37.2	.70	124.6	31.9	491
	" 11, "	3.82	37.9	.70	143.5	37.6	587
	Jan. 20, 1937	3.08	38.3	.69	113.0	36.7	534
11	Nov. 24, 1936	3.63	37.2	.69	98.3	27.1	416
23	Nov. 20, 1936	2.32	37.2	.69	69.5	29.9	415
24	Nov. 20, 1936	2.71	35.7	.71	78.3	28.9	403
26	Nov. 20, 1936	2.58	37.4	.69	77.1	29.9	410
27	Nov. 20, 1936	3.04	35.7	.69	78.6	25.9	374

These variations in basal metabolism are noted in experiments objectively selected as having met all the prerequisite conditions for true basal measurements and hence must be interpreted as indicating considerable variability in the basal metabolism of the marmot. This lability in basal metabolism is not a new experience in the Nutrition Laboratory, for it has been noted in a pronounced manner with the large domestic ruminants.¹¹ The problem is, therefore, as to what value can be taken as representing the true basal

¹¹ Benedict, F. G., and E. G. Ritzman, Proc. Nat. Acad. Sci., 1935, 21, p. 304; Ritzman, E. G., and F. G. Benedict, Carnegie Inst. Wash. Pub. No. 494, 1938, p. 113.

metabolism of the marmot. In the selection of the value representing the basal metabolism of an animal one must bear in mind that this value has two important uses, one as a representation of the real minimum metabolism of the individual animal, and the second use is as a means of comparing the metabolism of one animal with that of other animals for which presumably the average minimum values have been selected. The method of selection is open to debate. The Nutrition Laboratory has been criticised in the use of its measurements on humans in assuming that two well-agreeing low period values on any given day may be accepted as representing the basal metabolism on that day. This selection is founded upon the principle that it is easy to obtain metabolism measurements above basal, because so many factors rapidly and intensely influence the metabolism, but that with a normal animal and with accurate technique it is impossible to obtain values below basal. It is true that with humans profound sleep does lower the metabolism about 10 per cent.¹² With animals it is almost inconceivable that values below basal could be obtained in different periods on the same day, or with the same animal on different days. Hence we see no reason for not applying the same procedure in selecting basal values for animals as is used with humans, provided the experimental technique is above reproach. We have, therefore, in our selections of the basal values for our marmots been guided by the principle that two or three reasonably well-agreeing low period values on any one day may be averaged to represent the minimum basal metabolism of the particular animal on that day.

The pronounced differences in the heat production of the individual marmot from day to day, even on this basis of the use of minimum period values, raise the question as to what can be considered to be the *average* basal metabolism of each animal. That these differences, especially in the direction of high values, must be in large part due to a greater degree of muscular activity which is not indicated by the kymograph records, to a greater degree of tension or apprehensiveness, is highly probable, for in many instances marmots with high values ultimately showed lower values. For example, marmot I had a heat production of 654 and 664 calories in the first two experiments but in the last two experiments as low as 476 and 436 calories. With marmot W the successive values were 477, 600, and 441 calories. No. 9, however, had a constantly high metabolic level, only one value being below 446 calories and only two values below 489 calories in the entire series of eleven experiments. Similarly No. 10 has but two values below 490 calories. A general average of all these daily values for all 28 marmots has not been made, owing to the considerable variability in the metabolism of the same animal from day to day. An average would represent the average *basal* metabolism of the marmot as a species only when selected minimum values are determined for each animal. Can such an average properly be made? In table 6 have been summarized the averages of the minimum

¹² Mason, E. D., and F. G. Benedict, Am. Journ. Physiol., 1934, **108**, p. 377.

basal metabolism values noted on different days with the individual marmots, these averages representing in each case the minimum daily values indicated in italics in table 5. Only in the case of the last five marmots upon which but one experiment was made is the average based upon a one-day experiment. In all other cases minimum values on at least two and frequently three days were averaged, based upon the concept mentioned previously that it is impossible to secure measurements below the basal level.

TABLE 6—*Minimum heat production of the non-hibernating marmot*

Marmot	Body weight kg.	Rectal temperature ¹ °C.	Heat production per $10w^{\frac{1}{4}}$ per 24 hours cal.
A	1.49	37.7	440
B	2.18	37.2	500
H	2.79	38.3	470
I	2.43	38.4	470
K	1.62	36.7	440
M	2.55	480
R	2.61	34.3	390
S	2.95	36.6	350
U	1.72	37.0	350
V	1.96	36.6	² (560)
W	1.51	36.6	460
X	3.51	36.0	370
Y	2.39	36.6	340
1	3.23	36.5	310
2	2.60	37.2	390
3	3.09	36.7	380
4	2.78	36.0	290
5	2.60	37.6	390
6	2.24	36.6	370
7	3.46	37.1	400
8	2.06	38.0	460
9	3.46	37.8	440
10	4.58	38.1	470
11	3.63	37.2	420
23	2.32	37.2	420
24	2.71	35.7	400
26	2.58	37.4	410
27	3.04	35.7	370
Avg.....	2.65	36.9	410

¹ Average of all the rectal temperatures recorded for the marmot in table 5, see pages 57 to 59.

² Not included in average.

These averages in table 6 are practically all under 500 calories per $10w^{\frac{1}{4}}$, which is an extraordinarily low metabolism for any warm-blooded animal. To be sure, with mice values as low as this have been found, but the marmot is an animal whose weight is 100-fold the weight of the mouse and yet its heat production per $10w^{\frac{1}{4}}$ is very low. Of the entire series of 28 marmots one only has a value above 500 calories. This is animal V, the average for which is based upon only two experiments which were made only a few days after it arrived at the Laboratory. A general inspection of the course of the daily values in table 5 shows that occasionally habituation to the experiments plays a rôle and that the higher values with any one animal are

found at the beginning of the series of experiments. It can logically be contended that if marmot V had been studied for a longer period, lower values would ultimately have been found. Marmots 23, 24, 26, and 27, for example, had been living at the Laboratory for several weeks prior to their tests. Each of them was studied at about the same time and, although for only one day, the highest metabolism of any of the four was only 420 calories. The other instances in which high values for metabolism were found usually occurred a few days after the receipt of the marmots at the Laboratory. Next to animal V the marmot having the highest average minimum basal metabolism is animal B, with a heat production of 500 calories based upon only two well-agreeing daily values, the lower of which is 463 calories. Basal levels of 500 to 600 calories were frequently found on individual animals on individual days, but the selection of the lowest daily basal values for averaging leaves only one marmot in the series with an average minimum metabolism above 500 calories. One of the marmots, No. 4, has an extraordinarily low level of 290 calories. This is based upon an average of the last three experiments recorded in table 5. It seems inconceivable that a warm-blooded animal could have a metabolism as low as this. Yet other marmots on individual days had a minimum heat production for the day of under 300 calories. Thus No. 1 on November 4 had a metabolism of 292 calories and X on January 3, 296 calories.

This variability in the entire group from slightly under 300 to over 560 calories is not explained on the grounds of body temperature. On many of the individual days with different marmots occasionally somewhat high rectal temperatures were noted, but this was by no means invariably the case. Marmot V, for example, with a high heat production of 567 calories on October 15 had a low rectal temperature of 35.0° C., although on October 23 with a heat production of 560 calories it had a rectal temperature of 38.1° C. In the four instances when values under 300 calories were found, the rectal temperatures ranged from 34.0° to 37.5° C. (No. 1, 37.5° and 292 calories; No. 4, 34.0° and 276 calories; No. 4, 36.4° and 285 calories; X, 34.2° and 296 calories). In two of these cases low rectal temperatures under 35° accompanied the low heat production. On the other hand, in the other two cases the rectal temperatures were over 36° C. Thus it would appear that a low value for heat production may be obtained even with a reasonably high rectal temperature. A reservation should be made in this type of comparison, however, for it should be clearly recognized that the capturing of these animals and the insertion of the rectal thermometer did without doubt tend to increase somewhat the rectal temperature. That this procedure does not invariably result in this effect is likewise shown. From the data obtained, therefore, it is difficult to ascribe the low values of 300 calories and under solely to accompanying low rectal temperatures. Similarly, high metabolism values were occasionally obtained accompanied by rectal temperatures of 38° C. or over, which would lead one to believe that there is a correlation between these high temperatures and high metabolism. A sufficient number

of exceptions, however, are present to make it advisable to avoid any dogmatic statement as to the relative association of high body temperature and prevailing high metabolism.

These marmots varied considerably in size, ranging in average weight during the period in which the basal metabolism was measured from 1.04 kg. (U on October 12) to 4.80 kg. (No. 10 on September 21). The maximum weight (with No. 10) represents one of the days of low metabolism whereas the minimum (with U) represents almost the highest metabolism measured with the animal. In some instances even with one and the same animal there were considerable variations in weight. It so happens, however, that only rarely were the daily values selected as representing the minimum metabolism accompanied by great changes in body weight, the widest variations in weight on days when the lowest heat values were selected being usually only about 10 per cent. In at least one case there was a pronounced increase in weight during the period when the basal measurements were made. Thus marmot U had an average weight in the first three experiments of 1.05 kg. and an average heat production of 548 calories. On December 9, 18, 24, and 30, or essentially two months later its weight had increased 0.7 kg. or about 65 per cent and its metabolism had decreased to an average of about 380 calories. Marmot W on October 16 weighed 1.15 kg. and had a heat production of 477 calories. Its weight was 1.86 kg. on December 16, which represents an increase of 0.71 kg. or about 62 per cent, and its metabolism was slightly lower, 441 calories. No other pronounced increases in weight were noted with these animals during the basal metabolism study. With No. 10 instead of an increase in weight there was a decrease between September 21 and January 20, from 4.80 to 3.08 kg. On one of these days, the first, the metabolism was at the minimum basal level, 465 calories. On the last day, that is, on January 20, when No. 10 weighed 3.08 kg., its heat production was 534 calories. In these cases with striking changes in weight the general picture is that the metabolism per $10w^{\frac{3}{4}}$ is higher the smaller the body weight.

Large changes in body weight of one and the same marmot were not the rule in the relatively short periods of time that these animals were studied in the fall. The three most pronounced cases have just been discussed. A totally different picture was presented, however, in the spring after the marmots had been through a long period of fasting and hibernation and were then in the period of realimentation. At this time there was a rapid consumption of food. With the excess feeding the metabolism was whipped up to a high level, as the result of the regeneration of the body tissues and the body reserves. Under these conditions some of the marmots gained considerably in weight. During the winter, that is, from the time when measured in the fall to the time when they were refed in the spring, they lost at least from 1 to 2 kg. in body weight. At the time of the metabolism measurements in the spring they had gained about 0.4 kg. and hence were far from having regained their original weights. Under these conditions the

rectal temperatures of the marmots in all cases were higher than they were in the fall, other conditions being the same. In the fall at an environmental temperature of 28° the rectal temperature was approximately 37°, but in the spring at the same environmental temperature it was more nearly 38.5° C. The heat production was in all cases measurably higher in the spring, as is shown by studies made with nine marmots, of which unfortunately three were not measured in the fall. These three, O, P, and Q, arrived at the Laboratory in the spring and at this time were characterized by a high metabolism compared with the metabolism of those measured in the fall.

Marmot	Calories per 10w [‡]		Basal during spring refeeding level
	Fall basal	
B	500	590	
O	...	630	
P	...	450	
Q	...	600	
1	310	540	
2	390	610	
4	290	530	
9	440	670	
11	420	690	
Avg	390	590	

The average (not weighted) of the fall values is 390 calories and the average for the spring or refeeding level is 590 calories. Eliminating the measurements on O, P, and Q will but slightly raise the spring or refeeding level to 605 calories. Therefore, to the already observed fact that marmots even in relatively short periods have a variable basal metabolism must be added the fact that over a long series of months the variability may be even greater, although in this latter case the variability may well be accounted for by the difference in the state of nutrition and the extreme metabolic activity of what might be termed convalescence. Furthermore, although undoubtedly a large part of the increased intensity of the metabolism may be incidental to refeeding, season may also be a factor here, as these basal measurements during the refeeding period were all made in the spring and the initial basal measurements were made during the fall.

The discussion above deals solely with the heat production per 10w[‡]. The values for the total heat production and the heat production per kilogram of body weight are presented in tabular form in table 5 but need no special discussion. The well-known relationship between body weight and heat production, namely, that the heat production per kilogram is smaller the larger the body weight, is shown in nearly every case, although for such comparisons obviously the strictest basis for comparison, with all other variables eliminated, should be maintained. In the last analysis this strictest basis for comparison can be found only in those data italicized in table 5 as representing the true basal level. These values are frequently separated by a short period of time and are accompanied by such small changes in weight that the correlation between the body weight and the heat production per kilogram is not so strikingly brought out.

Average basal metabolism

In the selection of the values to be included in any average derived to represent the basal metabolism of the marmot as a species there are two steps to be followed. In the first place, for each day's experiment on any marmot one should select the minimum period values, based on the common practice of the Nutrition Laboratory of using at least two and preferably more well-agreeing low periods, thus automatically rejecting the high ones and ruling out periods of activity. Thus an average minimum value is obtained for each experimental day. In the reporting of the average minimum basal metabolism of each marmot in table 6 there is a further selection, in that the same principle applied in the averaging of the lowest period values for the day is again applied and the final average for each animal is an average of at least two and preferably more well-agreeing minimum daily values. As it has been clearly demonstrated that there is a lability in the metabolism of one and the same marmot from day to day, it is recognized that the minimum daily values must approach the true basal level. The higher values as well as the minimum values may have been obtained under conditions meeting all the prerequisites for basal measurements, but the lability in the basal metabolism (noted already in other animals) indicates that there is an internal stimulus to metabolism which has raised what might be termed the irreducible minimum level.

The average minimum heat production of each of our non-hibernating marmots, determined as above explained, is recorded in the fourth column of table 6. The maximum average value on this basis is 560 calories with marmot V, which unfortunately represents only two days of well-agreeing measurements, and the minimum is 290 calories with No. 4. The average for No. 4 is the average of three well-agreeing daily values, 308, 276, and 285 calories, and hence may be considered to be well-established. The fact that marmot V is the only one having a basal metabolism above 500 calories suggests that this animal was an exception. In view of the ease with which values above the true basal level can be obtained, this high metabolism probably represents lack of habituation. The average value for the other 27 marmots (that is, omitting V) is 410 calories.

The marmots varied in weight at the time of measurement from 1.49 to 4.58 kg., and the average weight of all 28 animals was 2.65 kg. It is a question whether or not this can be accepted as the average weight of the adult marmot, however, for it is believed that some of these animals were young. There were five marmots that weighed 3.46 kg. or over. The heat production of these five was, on the average, 420 calories. At the other extreme there were five marmots weighing under 2 kg., one of these being V, the animal already mentioned as having a high metabolism. If we confine ourselves to the four animals weighing 1.72 kg. or under we find that the average heat production of these four is 420 calories. Thus it would appear as if the metabolism of the extremely heavy marmots did not differ from that of

the extremely light-weight marmots, in other words, that the heat production per $10w^{\frac{1}{2}}$ was essentially uniform throughout the entire series.

The striking feature of this study is the extraordinarily low metabolic level found with these marmots. Even the highest value of 560 calories with V represents a level of metabolism never before recorded for any other warm-blooded animal weighing in the neighborhood of 2.5 kg.¹³ The extremely low value for No. 4 of 290 calories is hardly approached even by the small, heterothermic white mouse. It is clear that the marmot (which belongs to that class of animals that can at times enter a profound lethargy ultimately ending in deep hibernation, when the temperature regulation is thoroughly disorganized) has an extremely labile metabolism and labile rectal temperature, but that when non-hibernating and having an average rectal temperature of 36.9° C. its basal heat production averages 410 calories per $10w^{\frac{1}{2}}$. This figure will serve as a baseline not only representative of the average basal heat production of the average marmot, but more especially for comparison with the metabolism of the marmot in its various stages of lethargy and in deep hibernation. However, although the average value of 410 calories is presented as the basal metabolism of the marmot, it should not be overlooked that five animals had values of 350 calories and below, and two of them 310 and 290 calories, respectively. These data offer certainly a clear indication of variability even in the minimum basal metabolism of different marmots.

WATER VAPORIZED FROM THE LUNGS AND SKIN

One of the measurements accompanying nearly all the metabolism studies on animals at the Nutrition Laboratory is that of the water vaporized from the lungs and skin. This measurement has a physiological interest of its own but is of special interest in energy studies, for the vaporization of water represents one of the paths of loss of heat from the body. In order to make such measurements accurately, the water vaporized in the respiration chamber must be derived solely from the lungs and skin and must not include any water vaporized from urine and feces. Furthermore the respiration chamber itself should not be sealed with a water seal. As a matter of fact, in all experiments in which water-vapor determinations were made on our marmots the respiration chamber was hermetically sealed with wax. If along with the water-vapor determinations the gaseous metabolism is measured at the same time, one can compute the heat production and calculate what proportion of the heat was lost in the form of water vapor. The optimum condition for using the open-circuit respiration apparatus with the Carpenter gas-analysis apparatus is to ventilate the chamber at such a rate that the air leaving the chamber should have a carbon-dioxide content of not far from one per cent. The rate of ventilation necessary to maintain the carbon-dioxide content at this level frequently does not remove from the

¹³ Dontcheff and Kayser (Dontcheff, L., and C. Kayser, Compt. Rend. Soc. de Biol., 1936, 122, p. 328) report approximately this value for the non-hibernating marmot.

chamber all the water vapor given off by the animal. Often under such conditions there would be a condensation of water on the inside of the chamber and in the pipes leaving the chamber. It is impractical to increase the total rate of ventilation, for by so doing the percentage of carbon dioxide in the outgoing air would be lowered and thus the slight, although frequently present, error in the gas analysis would be exaggerated. The other alternative is to introduce a supplementary absorbing train into the system, whereby the air leaving the chamber would first pass through a drying reagent and then be delivered, dried, back into the chamber at the intake end. By so doing it is possible to take out and weigh in receptacles large amounts of water vapor and at the same time the carbon-dioxide percentage of the air current will not be altered. This device is shown in figure 2 (page 16).

Many of our water-vapor experiments were lost, due to passage of urine or feces which is obviously not controllable by the observer. Fifteen animals, however, were successfully measured at an environmental temperature

TABLE 7—*Water-vapor output of the non-hibernating marmot*

Marmot and date	Body weight	Environmental temperature	Water vaporized per 24 hours		Heat lost in water vapor
			Total	Per kg.	
S		kg.	°C.	gm.	gm.
Oct. 22, 1935	2.98		28	25.1	8.4
Dec. 4, "	3.43		28	34.1	10.0
" 11, "	3.35		16	26.4	7.9
" " "	3.35		16	22.9	6.8
W					p. ct.
Oct. 24, 1935	1.32		.28	31.7	24.0
" " "	1.32		28	24.7	18.7
Dec. 11, "	1.82		16	23.3	12.8
" " "	1.82		16	19.9	10.9

of 28° and likewise at 16° C. Typical results are presented in table 7 for marmots S and W which were fasting 24 hours. These values serve not only as basal values for the non-hibernating marmot but particularly for comparison with subsequent measurements made on the marmot in deep hibernation.

Marmot S weighed, on the average, more than twice as much as W. The total amount of water vapor given off by S per 24 hours at 28° C. was not far from 30 gm. At 16° both the total amount and the amount per kilogram were slightly lower. As 1 gram of water requires for its vaporization 0.585 calorie,¹⁴ one can easily compute how many calories are lost in this form and from the total caloric production based on the measured oxygen consumption can compute the percentage of heat lost in the vaporization of water. This is recorded in the last column of table 7. At 28° the heat lost

¹⁴ Smith (Physical Review, 1907, 25, p. 145) shows that 0.585 calorie is the heat of evaporation of water at 20°C. At 35° and 40°C., or more nearly the body temperature of the non-hibernating marmot, the values would be 0.577 and 0.574 calorie, respectively, or about 2 per cent lower.

through this path by S averaged 18 per cent, but at 16° it fell to an average of 7.8 per cent.

With animal W the decrease in the total amount of water vaporized at 16° as compared with that at 28° C. was slightly more pronounced than in the case of S. As there was an increase of nearly 40 per cent in the body weight of W between the two experiments, it follows that its water-vapor output per kilogram was much less at the lower environmental temperature, which accentuates the finding with S. One of the most striking features of table 7 is the fact that W, weighing half as much as S, could give off under equal conditions of environmental temperature essentially the same total amount of water vapor as the larger animal.

With W the heat lost by vaporization of water at 28° averaged 21 per cent as compared with the 18 per cent lost by marmot S at 28°, and at 16° it fell to 7.8 per cent or precisely that found with S. As will be shown later (page 69), the metabolism of the non-hibernating marmot is greatly increased at 16° C. Hence, because the total water-vapor output, although somewhat decreased at 16° over that at 28°, is much the same at both temperatures, it follows that the lower percentage loss of heat by the path of water vapor at 16° (which is only about one-half or one-third at 16° what it is at 28°) is explained in large part by the greatly increased heat production and not so much by the decrease in the water vaporized, although there is a definite decrease in the water-vapor output expressed per kilogram of body weight.

Accurate measurements of the water vapor given off by various animals are, for the most part, lacking because so many experiments are complicated by the voiding of urine or feces in the chamber. With humans it is assumed that under ordinary conditions of basal metabolism measurement approximately 25 per cent of the total heat production is lost through the path of water vapor. At 28° C. marmots S and W lost not far from this same percentage of heat in this manner (20 per cent, on the average). When it is considered that man has no natural protective covering and that there is a rapid diffusion of moisture through his porous clothing, whereas the marmot has a dense fur covering, the fact that under basal conditions the proportion of heat lost in the vaporization of water is almost exactly the same with marmots and humans is particularly striking.

Although based upon an entirely different method of measurement, the water-vapor output of these two marmots as directly measured confirms in a general way our findings on the insensible perspiration of the non-hibernating marmot. The insensible perspiration, however, always includes a certain proportion of carbon from the body and thus, theoretically at least, should be somewhat larger (about 15 per cent¹⁵) than the water-vapor output itself.

The fact that at 28° C. the non-hibernating marmot loses essentially the same proportion of heat in the vaporization of water as do many other ani-

¹⁵ Benedict, F. G., and C. G. Benedict, Biochem. Zeitschr., 1927, 186, p. 278; *idem*, Proc. Nat. Acad. Sci., 1927, 13, p. 364.

mals, including man, is an indication that the marmot is adequately provided with a mechanism for losing heat by vaporization of water which functions under normal conditions (that is, in repose) precisely as do such mechanisms of most other animals. With increased muscular activity and increased heat production, in the case of man and other animals, the amount of heat lost by vaporization of water increases enormously. We have no doubt that this is true also of the marmot. The fact, however, that the marmot has a heat-losing mechanism as perfectly developed for normal resting conditions as have other animals is of special note when one considers the extraordinarily low total heat production of this animal. The marmot's body temperature is essentially that of most other animals. Hence from the standpoint of temperature potential between body temperature and environmental temperature it should lose heat per unit of surface area exactly as animals of the same body temperature. In view of the low heat production of the marmot, however, one might have expected that its percentage loss of heat by vaporization of water would be considerably lower than that noted with other warm-blooded animals, in other words, that this path for heat loss would be only imperfectly used. No evidence is available as to the true skin temperature of the marmot. Such measurements made over the fur or at the base of the hairs might give a much lower skin temperature than that of other furred animals and thus explain that the loss of heat to the environment is correspondingly lowered to compensate for the extremely low metabolism itself. These data are not available, but the fact that under conditions of basal metabolism measurement the percentage loss of heat through the path of water vapor is essentially the same with the non-hibernating marmot as with other animals is, in light of the marmot's extremely low metabolism, at least most challenging and worthy of further study.

METABOLIC REACTION TO ENVIRONMENTAL TEMPERATURE

Metabolism at 16° C.

One of the objects of this study of the non-hibernating marmot was to secure data for the interspecific comparison of various animals not only at thermic neutrality but likewise at the "laboratory temperature" selected by Rubner for his classic study of different animals, from which the law of surface area as applied to various species was conceived. Hence, in accordance with the custom of measuring all animals at the Nutrition Laboratory, this investigation on marmots included not only a series of metabolism measurements at thermic neutrality but also a series at 16° C. From the preliminary survey of the probable extent of the zone of thermic neutrality, in which a profound influence of small deviations from the critical temperature was noted, it is natural to expect a great influence upon heat production of a lowering of the environmental temperature to 10°, 12°, or 16° C.

In table 8 are summarized the results of measurements upon 15 non-hibernating marmots that had been kept at 16° for 24 hours or more and had

been without food for from 24 to 48 hours. Discussion has already been given of the fact that the subjecting of these animals to an environmental temperature of 16° resulted invariably in a lowering of the rectal temperature. All but one of the rectal temperatures in this series, which were

TABLE 8—*Metabolism of non-hibernating marmots at 16° C.*¹

Marmot	Date	Body weight kg.	Rectal tem- perature °C.	Heat production per 24 hours		
				Total cal.	Per kg. cal.	Per 10w ² cal.
A	Nov. 6, 1931	1.47	35.0	79.7	54.1	613
B	Oct. 21, 1931	1.76	36.0	96.1	54.7	758
	Nov. 4, "	2.10	35.6	106.0	50.6	646
D	Oct. 13, 1932	1.47	35.4	108.3	73.5	833
	Nov. 10, "	1.67	34.3	132.4	79.2	939
	Dec. 7, "	1.28	31.5	113.5	88.9	962
E	Oct. 26, 1932	2.47	36.3	166.0	67.4	907
	Nov. 8, "	2.50	34.8	145.0	58.1	788
	" 17, "	2.44	33.9	172.2	70.6	951
	Dec. 6, "	2.69	33.6	169.5	64.2	891
F	Nov. 9, 1932	1.76	34.4	141.7	80.6	970
	Dec. 8, "	1.81	35.0	129.7	71.5	871
G	Nov. 2, 1932	1.11	35.5	103.3	93.4	965
	" 12, "	1.11	37.1	117.5	106.2	1098
H	Jan. 12, 1934	2.78		203.0	72.9	1023
I	Jan. 10, 1934	2.38		206.0	86.7	1159
M	May 3, 1935	2.57		196.4	76.4	1047
M	" 8, "	2.53		160.5	63.6	866
R	Dec. 6, 1935	2.71		194.0	71.7	991
S	Dec. 11, 1935	3.35		162.0	48.2	721
	" 27, "	3.31		181.0	54.5	813
	" 31, "	3.30	35.7	174.0	52.8	786
U	Dec. 13, 1935	1.71		94.0	54.7	659
	" 20, "	1.61		112.0	69.3	813
W	Dec. 11, 1935	1.82		143.0	78.5	959
X	Dec. 6, 1935	3.34		165.0	49.4	739
	" 31, "	3.44	35.7	182.0	53.0	799
Y	Dec. 13, 1935	2.35		198.0	83.7	1119
	" 19, "	2.38		200.0	84.1	1122
	Jan. 2, 1936	2.31	34.8	164.0	70.9	938

¹ Living at 16° C. for at least 24 hours.

determined after the respiration experiments ended, were at a lower level than the average of 36.9° C. recorded in table 6 for the basal condition. With G on November 12 the highest rectal temperature in the series, 37.1° C., was noted, but with the other marmots the temperatures were frequently

under 35°. Accordingly one would suspect that these marmots are partially poikilothermic in nature and would tend to have a lowered metabolism at this lower cell temperature. A momentary inspection of the values for the heat production, particularly expressed as calories per $10w^{\frac{1}{3}}$, shows, however, that this is far from the case. The lowering of the rectal temperature, which would be expected to lower the heat production, is far more than offset by the stimulus of the low environmental temperature to heat production. The heat values range from 613 calories on one day with marmot A to 1159 calories with marmot I. An undue amount of time could not be devoted to this special study at 16°, and hence the actual number of days when such observations were made is far less than was the case when the basal metabolism was determined. Comparison with the clearly established basal metabolism of the marmot leaves no doubt but that the effect of an environmental temperature of 16° is to increase the metabolism and that this increase is accompanied in nearly every case by a subnormal body temperature.

The variability in the heat production at 16° C. is considerable, relatively far more so than in the basal metabolism series. This is to be expected, for these animals had been stimulated by the low environmental temperature, and although every attempt was made to rule out activity, the general impression obtained from the kymograph curves is that the marmots were by no means so tranquil as at the higher temperatures. The averaging of such a wide series of values as given in table 8 is debatable. If such is done, however, the average value is 899 calories. As the average basal value has already been shown to be 410 calories, the conclusion is that the low temperature of 16° has more than doubled the metabolism. In other words, a lowering of the environmental temperature twelve degrees (from 28° to 16° C.) has resulted in an increase in the metabolism of 119 per cent or 9.8 per cent per degree. This effect is not to be confused with any possible effect of the decrease in the rectal temperature. This is solely an effect of environmental temperature, unfortunately not perfectly disassociated from physical activity.

Effect of rapid changes in environmental temperature

It has already been noted (page 53) in the study of the zone of thermic neutrality and the critical temperature that the marmot rapidly adjusts itself to environmental situations and that there is no need for the long period of adaptation suggested as necessary for smaller animals. The rapidity of adjustment was especially studied in a series of experiments made with eight marmots on 15 days. After each animal had been living beforehand at 28° for 24 hours and fasting during this time, it was measured in the apparatus at 28°, then the chamber was quickly cooled, and the animal was studied at 16° C. In such comparisons the attempt was made to secure experiments in which the activity was the same at both temperature levels, so that the measurements would not be complicated by differences in activity. As already stated in discussing earlier tables, it is almost invariably true that

marmots at 16° are not so tranquil as at 28°. For this comparison of the effect of rapid changes in environmental temperature, therefore, we have selected from the measurements made at 28° and likewise from those made at 16° the lowest two or three period values on each day. The averages of these low values are summarized in table 9. An increase in metabolism when the environmental temperature was lowered to 16° is noticeable in every case. The smallest effect was with X, studied in only one experiment, but even here the increase (from 405 to 632 calories) was over 55 per cent. The average value for all the marmots at 28° was 432 calories. The average for all the marmots at 16° was 987 calories. In general, therefore, the increase was 127 per cent.

TABLE 9—*Effect of change in environmental temperature (from 28° to 16°) upon metabolism of non-hibernating marmots*

Marmot	Date	Body weight	Heat production per $10w^{\frac{3}{2}}$ per 24 hours		Rectal temperature at 16° C.
			At 28° C. ¹	At 16° C. ²	
H	Jan. 9	2.89	481	842	34.2
I	Jan. 16	2.47	436	1162	35.4
M	April 22 " 25 " 30	2.41 2.59 2.61	546 474 531	1370 1350 1267	37.3 37.8
R	Dec. 16	2.89	480	1138	34.7
S	Oct. 29 Dec. 18	2.99 3.36	447 432	762 814	35.5 35.8
U	Dec. 18 " 24 " 30	1.73 1.71 1.76	343 357 414	1011 997 945	36.7 36.5 36.9
X	Dec. 17	3.51	405	632	33.9
Y	Dec. 17 " 23 " 30	2.42 2.38 2.36	350 322 343	1197 1043 831	36.5 35.9 34.9

¹ Marmots had been without food and at 28° for 24 hours before experiments.

² About one hour after measurements at 28° C.

Comparison of the average metabolism at 16° when the marmots had been for at least 24 hours at this temperature and the average basal metabolism measured at 28° has already shown that the sojourn at 16° resulted in an increase in metabolism of 119 per cent. The rapid transition from 28° to 16° C., however, caused an increase averaging 128 per cent. In other words, the sudden change to 16° resulted in a 9 per cent greater increase in metabolism than did the longer habituation at 16°.

A complementary study was made of marmots that had been living and measured at 16° and were suddenly changed to 28°. All such measurements are complicated by the differences, oftentimes small but not infrequently

great, in muscular activity at the two temperatures. In table 10 are given the data obtained on those marmots that had been living at least 24 hours at 16° and were measured first at this temperature after a 24-hour fast, and subsequently after a rapid change to 28° C. These animals that were specially used for this comparison of the transition from 16° to 28° were likewise included in table 8 among the marmots measured at 16°. The particular value of repeating in table 10 the data secured at 16° is that they are directly comparable with the measurements at 28° C. Each marmot showed a pronounced decrease in metabolism upon entering the 28°-temperature phase. The average value for all the animals at 16° is 937 calories. This value is somewhat higher than that (899 calories) derived from the data in table 8,

TABLE 10—*Effect of change in environmental temperature (from 16° to 28°) upon metabolism of non-hibernating marmots*

Marmot	Date	Body weight	Heat production per $10w^{\frac{3}{4}}$ per 24 hours		Rectal temperature at 28° C.
			At 16° C. ¹	At 28° C. ²	
H	Jan. 12	2.78	1023	488	36.1
I	Jan. 10	2.38	1159	476	36.3
R	Dec. 6	2.71	991	581	35.3
S	Dec. 11	3.35	721	333	35.4
	" 27	3.31	813	313	33.9
U	Dec. 13	1.71	659	421	35.2
	" 20	1.61	813	376	35.6
W	Dec. 11	1.82	959	406	34.9
X	Dec. 6	3.34	739	404	35.4
Y	Dec. 13	2.35	1119	332	35.8
	" 19	2.38	1122	446	36.2

¹ Marmots had been without food and at 16° for 24 hours before experiments.

² About one hour after measurements at 16° C.

as the marmots A and B with lowest values at 16° do not appear in this subsequent study. The average of the measurements at 28° for the animals listed in table 10 is 433 calories. As the basal value for the entire series of marmots is 410 calories, it can be seen that this rapid adjustment of but an hour at 28° has succeeded in lowering the metabolism of these animals from an average level of 937 calories to approximately the average basal level noted for the entire group.

In a few specific cases in table 10 the metabolism at the 28° level is lower than the minimum value selected for the animal on the basis of several days and reported in the summary table 6. With marmot S, for example, the heat production after the rapid change to 28° C. averaged 323 calories as compared with the average of 350 calories given in table 6. Thus for the

moment one could argue that this sudden change from the severe cold to warmth had resulted in an actual lowering of the basal metabolism of this animal. This phenomenon is likewise exhibited by marmot W, with which an average value of 406 calories was noted on December 11 in four periods. As a matter of fact, two periods which might be averaged gave values of 333 and 366 calories. The average minimum selected for W on the basis of these two periods would be 350 calories or 110 calories lower than the minimum selected as the basal metabolism of this animal, *i.e.*, 460 calories (table 6). This minimum basal selection was based upon the condition that the animal should be 24 hours at the temperature at which it was to be measured, although it was shown by a number of experiments that the previous environmental temperature did not profoundly affect the metabolism at thermic neutrality.

In two other instances this same phenomenon was noted, marmot Y on December 13 having an average heat production of 332 calories or slightly lower than the selected basal value of 340 calories, and marmot X on December 6 actually having one period value (332 calories) lower than the selected basal value.¹⁶ This phenomenon has not infrequently been met in the Nutrition Laboratory's experience. It is puzzling and not yet wholly explainable. One of the most astounding experiences of this type was with certain steers in the investigation at Durham in collaboration with Professor E. G. Ritzman. At least two different steers, when brought from a very cold barn and placed in a warm respiration chamber, had the lowest heat production ever measured with these great ruminants.¹⁷ That there may be some over-compensation in some cases seems to be probable. The precise nature of this over-compensation is not clear, and it certainly is not explained in our own experiments.

Metabolism at 10° C.

The tremendous stimulation of a cold environment incidental to a sojourn and measurement at a low environmental temperature is further shown by experiments on two marmots, O and Q, that were studied under the unusual conditions of cold in summer, being placed in a refrigerator suitably ventilated for these observations. With Q three experiments were made at an environmental temperature of 10° C. Although its activity was by no means as insignificant as it was at 28°, nevertheless it was not great enough to account for the increased metabolism, which averaged for the three different

¹⁶ As basal values for these particular marmots obtained under more nearly ideal temperature conditions are available, we have not introduced into the basal table 6 the values found under these special conditions of rapid transition in temperature. Subsequently special experiments (with animals Nos. 23, 24, 26, and 27) with extreme variations in temperature from 8° to 28° were made, showing again no period of adaptation necessary, *i.e.*, essentially the same basal metabolism at the transition from 8° to 28° as obtained when marmots were living at 28°. These latter values were therefore included in the basal table 6, as no other basal values were available for these animals.

¹⁷ Benedict, F. G., and E. G. Ritzman, Carnegie Inst. Wash. Pub. No. 377, 1927, p. 218.

days at 10° about 1450 calories. As the basal metabolism of this marmot obtained just prior to this time was 433 calories, the increment at 10° (*i.e.*, a decrease of 18° from 28°) corresponds to 1000 calories or nearly 250 per cent, which means an increase of about 14 per cent per degree. It has already been noted that at 16° the increase in metabolism is at the rate of 8 per cent per degree. With marmot O an experiment at 10° gave a heat production of about 1500 calories. This animal showed in the rather scanty measurements possible with it (for it was obtained late in the spring) a much higher basal metabolism than the normal range, *i.e.*, somewhat over 600 calories. In this experiment at 10° the increase was 150 per cent, or only 8 per cent per degree. When the environmental temperature was lowered still further to an average of 6.8°, the heat production was not increased, for the average for the five periods (which varied considerably) was slightly under, rather than over, 1500 calories.

In all these experiments, both with O and Q, the rectal temperatures at the end of the experiments were well within normal limits. The lowest observed on one day with Q was 35.6°. The highest was 38.9° with animal O on the day when the environmental temperature was but 6.7° C.

If the lowest period values on these days at low temperatures are selected, the heat production on the first of the three days with Q was 1210 calories, on the second day 1320 calories, and on the third day 1340 calories. With animal O at the 10°-environment the two lowest periods averaged 1400 calories, and at the exposure to 6.8° the two lowest period values averaged 1240 calories. Thus the specific influence of the extreme cold even with these marmots that are unusually well protected by subcutaneous fat and by fur is fully as pronounced as with any animals that the Nutrition Laboratory has studied.

To make a specific study of the influence of the cold environment *per se* it would be ideal to have an experiment in which the external muscular activity was the same both at the low and the higher temperature. It was noted, however, that the marmot was almost invariably somewhat more active in the cold than when under warm conditions. From our long experience in studying the effect of muscular activity of itself upon metabolism it is inconceivable to think that any appreciable percentage of the great increase in metabolism noted with the falling environmental temperature can be attributed to the relatively small increases noted in muscular activity. We are forced, therefore, to the conviction that in this case there is a *true chemical regulation* of heat production, *i.e.*, an increase in heat production to combat the lower environmental temperature.

PHYSIOLOGY OF THE NON-HIBERNATING MARMOT DURING PROLONGED FASTING

Among the physiological factors taken into consideration in the Nutrition Laboratory's studies on various animals have been the resistance to complete absence of food as indicated by the behaviour of the different bodily functions, the length of time the animal can withstand fasting before dying, and the rate of loss in weight during prolonged fasting. These are all important factors, knowledge concerning which contributes to the better understanding of the physiology of the normal animal. The special purpose of the research on the marmot was to study its physiology during the transition into hibernation, during hibernation, and during the transition out of hibernation. With this type of animal, observations during fasting in the awake condition unaccompanied by complications are difficult to secure. To be sure, many of our marmots went for long periods of time without food, but in most of these instances, in striking contrast to other fasting animals, the marmots frequently hibernated while fasting, living in a state of suspended animation with extremely low metabolism and hence extremely low drafts upon body material. Certain of the marmots fasted for a month or more without indications of approaching hibernation such as a marked lowering in rectal temperature and respiration rate, and the data obtained with these animals will serve for a study of the physiological changes experienced by the fasting, non-hibernating marmot.

LOSS IN BODY WEIGHT

One of the first factors to be observed in prolonged fasting experiments is the change in body weight which, as a result of no food intake and the continued drafts upon body material, usually is a steady decrease throughout the fast. The body weights of many animals have been studied over long periods of fasting, and the general course of the body-weight curve is not dissimilar in most cases. Because of the tendency of the marmot to hibernate, the influence of this factor upon the body weight during fasting must be continually kept in mind. The body weight changes of three marmots, H, I, and K, from day to day during a fasting period extending from approximately the middle of January until (with marmot K) the end of June have been plotted in figure 6. In this chart the body weights recorded on those days when the rectal temperature was 32° or below are indicated by hollow circles. The solid dots represent weights on days when the body temperature was above 32°C. This method of indicating the differences in body temperature may serve as a suggestion as to whether or no the marmots were in hibernation or at least had a tendency to hibernate.

The general trend of these curves of weight loss in fasting is similar to

those of Polimanti¹ appearing in a plate at the end of his book. On only a few days was the rectal temperature of H as low as 32°, and this animal may, therefore, be considered to have been fasting under normal conditions uncomplicated by periods of hibernation. Under this non-hibernating, fasting condition the rate of loss in body weight from the beginning to the end of the fast is expressed by a straight line. Marmot I had a rapid loss in weight until February 9, when subnormal body temperatures began to appear with accompanying hibernation. The rate of loss from then on was, for the most part, uniform and much slower, although after April 15, at which time hibernation ceased, the body temperature was somewhat higher and there was a slightly greater rate of loss in weight. With marmot K the rate of loss was fairly rapid at the very start, but subnormal rectal temperatures appeared as early

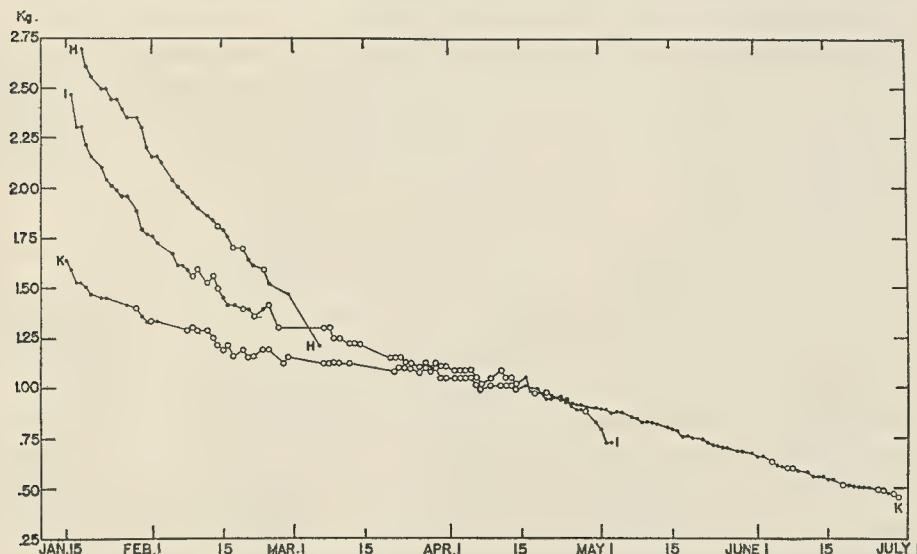


FIG. 6—CHANGES IN BODY WEIGHTS OF MARMOTS DURING PROLONGED FASTING

The solid dots represent weights on days when the rectal temperature was above 32°C. and the hollow circles, weights when the rectal temperature was 32°C. or below and the marmot may have been hibernating.

as January 29 and continued for the greater part of the time thereafter until about the middle of April. This animal, therefore, as well as marmot I, was hibernating for a considerable length of time during its fast.

The longest fast was that of K, from January 15 until June 29, or nearly six months. This was one of the smallest marmots, and hence it had the least body reserve for, although it was not fully grown at the start of the fast, it was six months older at the end.

The total losses in weight experienced by these marmots are of special interest. Marmot H began its fast with a weight of 2.92 kg. On the last day of observation, March 6, it weighed 1.22 kg. It therefore lost 58 per cent in weight in somewhat less than two months, and the rate of loss, as

¹ Polimanti, O., *Il Letargo*, Roma, 1912.

stated above, was fairly regular during this time. With marmot I the total loss in weight from the beginning to the end of its fast, which included a long period of very low metabolism during the hibernating stage, was 71 per cent. The animal started to fast with a body weight of 2.47 kg., and it had lost half of its weight on March 11. The remaining loss, that is, from March 11 to May 3, represented about one-fourth of its initial weight. Both H and I started fasting at nearly the same weight level, and until marmot I began to have subnormal temperatures, the rate of loss as shown by the parallelism of their body-weight curves was much the same. Marmot K was a much smaller animal and started fasting at a much lower weight level than did the other two marmots, but at the end of its fast nearly 6 months later its weight had decreased from an initial level of 1.64 kg. to 0.46 kg. The actual total loss was thus 72 per cent.

The effect of hibernation in retarding the weight loss is clearly shown by the curve for K. At the beginning of its fast there was a rapid loss in weight. With the onset of the lower body temperatures and hibernation the curve flattens out, but toward the end when hibernation ceased it again assumes a more rapid descent.

Although precise data with regard to the ability of 2- to 3-kg. animals to withstand fasting are not numerous, our survey of the literature shows that the marmot even prior to hibernation has a capacity to lose great amounts of body tissue without serious detriment. A number of species of animals that do not hibernate have been found to lose as much as 40 per cent of their body weight while fasting. Undoubtedly if these marmots had not had a very much lessened metabolic activity during the period of hibernation, they would have lost weight more rapidly than they did and would have died earlier. The non-hibernating marmot does not, therefore, differ strikingly from other animals in its resistance to fasting. This is perhaps a little surprising, for the basal metabolic rate of the marmot is lower than that of other animals, for example, nearly half that of the dog.

Although the absolute losses in weight are important when one considers the individual animal, these marmots varied considerably in body size and hence a strict comparison of the losses in weight in prolonged fasting is best made by considering the percentage losses rather than the absolute decreases. The three marmots already commented upon, H, I, and K, lost 58, 71, and 72 per cent, respectively. A number of other marmots were subjected to long fasts ranging from 77 to 173 days. The percentage losses in weight in these fasts, together with those of the three marmots already presented, are reported in table 11. The percentage losses vary from a minimum of 35.8 with marmot E, which fasted 100 days, to a maximum of 71.8 per cent with marmot K, already discussed, which had fasted 167 days.

LENGTH OF FAST

When one considers the number of days that these marmots fasted, as shown in table 11, one should ever bear in mind that there is a complication

in this picture. When the marmot is not hibernating, each fasting day may be said to be, roughly speaking, of equal value in its effect upon the metabolism, although there is a tendency for the actual total metabolism to decrease and for the body weight to diminish as the fast continues. If the marmot is kept at thermic neutrality, the metabolism would be essentially basal at the start of the fast. If the marmot is kept at a temperature much below thermic neutrality, for example, at 10°, its metabolism might be increased as much as 150 or 250 per cent, as we have already seen, or hibernation might set in. When hibernation sets in, there is a great decrease in metabolism to a level amounting to only a fraction of the normal basal value. Consequently if the fasting marmot hibernates on days when the environmental temperature is much lower than thermic neutrality, its metabolism may be so low on these days that the draft upon body material will be but a fraction of that when the fasting marmot is not hibernating. This fraction

TABLE 11—*Per cent weight lost by non-hibernating marmots during prolonged fasting*

Marmot	Weight at start of fast <i>kg.</i>	Weight at death <i>kg.</i>	Per cent loss	Days fasting
B	3.29	1.96	40.5	96
D	1.36	.87	36.4	77
E	2.72	1.76	35.8	100
F	1.81	.97	46.4	89
G	1.11	.51	54.2	99
H	2.92	1.22	58.3	48
I	2.47	.73	70.6	107
K	1.64	.46	71.8	167
L	2.27	.99	56.3	173
M	2.86	1.01	64.7	159
O	2.37	.97	59.1	83
Q	2.84	1.31	53.9	83
R	2.89	1.05	63.8	166
S	3.46	1.24	64.2	83
W	1.86	.84	54.8	131
X	3.66	1.38	62.3	142
Y	2.45	.77	68.6	107

will vary considerably. It may be only about one-tenth of the basal metabolism at 28°. Ten days of hibernation at 10° would, therefore, result in drafts upon body material equivalent to but one day's draft when the marmot is fasting at thermic neutrality. On the other hand, when the marmot, non-hibernating, fasts at a temperature of 10°, the body drafts during one day of such life may be the equivalent of the draft during about 30 days of hibernation at the same temperature. Hence the last column of table 11, in which is indicated the days fasting, can be properly interpreted only by taking this point into consideration.

Among those marmots that fasted 100 days or more, the minimum loss in weight was 35.8 per cent. This relatively small loss during a long fast is noted only with marmot E. Four of our animals fasted for 159 days or more, the longest fast being 173 days. There is not a good correlation between length of fast and weight loss. This is undoubtedly due to the fact that the ele-

ment of hibernation appeared with a number of these animals, which disturbs and accentuates the length of fasting, for hibernation reduces the drafts upon body material below the normal minimum.

POST-MORTEM EXAMINATION

We are indebted to Dr. Milton O. Lee for a post-mortem examination of marmot B which died on October 1, 1932, after fasting 96 days. The dead weight was 1956 grams. The animal, a male, had an excellent coat of fur, both "wool" and coarse hair. There was much fat between the skin and muscles. The muscle had a yellowish tinge. The retroperitoneal fat was considerable and the mesenteric fat was a great mass. There were considerable fat deposits in the omentum. The testes were extremely small and soft and were in the abdominal cavity. The lower part of the colon was empty, but the upper part contained much watery material. The cecum had watery fluid inside. The spleen was normal in color and of expected size. There was some fluid in the stomach. The liver was light yellowish brown in color. There was probably jaundice, as indicated by the yellow color of the fat and yellow color of the skin. The gall bladder was greatly enlarged, ball shaped, and about $2\frac{1}{2}$ cm. in diameter. The liver was firm with no obvious abnormality except in color. There were no adhesions. The kidneys and lungs appeared perfectly normal. The heart was probably normal in size, but the adrenals were rather small, although it is not quite certain that the whole of them were removed. The adrenals, kidneys, heart, thyroid, gall bladder, liver, testes, and spleen were weighed separately and the weights were as follows:

Kidneys	15.23 gm.
Spleen	3.77
Heart	8.81
Testes61
Thyroid40
Adrenals23
Liver	87.63
Gall bladder	8.70

The length from nose to anus was 41.7 cm.

No obvious cause of death was suggested by the post-mortem examination, *i.e.*, no obvious pathology to account for it. The color of the liver, the pronounced yellow color of the body fat, the skin, and the light yellow color of the muscles would suggest liver injury and escape of bile into circulation—all consistent with a long period of starvation.

INSENSIBLE PERSPIRATION

Several fasting marmots were placed upon the balance and accurately weighed from period to period, usually at environmental temperatures between 21° and 29° , averaging for the most part about 26° C. The insensible losses in weight, which were measured usually for several consecutive periods, are recorded in table 12 as the average loss per hour for the entire day. As

the marmots all had much the same weights, varying only from 0.85 to 1.28 kg., the losses have not been expressed per kilogram per hour. The details of a typical experiment with marmot R on May 26, when the insensible perspiration was determined in seven consecutive weighings, are given in table 13. The loss per hour was large in the first period (a fact not infrequently noted with other animals) possibly due to moisture around the anus or on the fur from previous urination. Thereafter the loss in weight was fairly uniform.

TABLE 12—*Insensible perspiration and respiration rate of fasting marmots*

Marmot	Date	Body weight	Days fasting	Length of period	Environmental temperature	Average insensible loss per hour	Respiration rate per minute
R	1936	kg.		min.	°C.	gm.	
	May 25	1.15	162	164	27	0.66	
Y	" 26	1.15	163	439	26	.54	4
	May 26	1.20	82	441	26	.45	
	" 28	1.17	84	460	21	.31	
	" 29	1.14	85	107	23	.46	
	June 2	1.09	89	122	27	.36	
	" 3		90	296	29	.26	28
	" 5		92	375	28	.26	44
	" 6	1.01	93	183	26	.29	31
	" 8		95	119	26	.30	5
	" 16	.85	103	201	24	.55	5
S	May 25	1.28	81	157	28	.60	
	" 26		82	162	24	.33	8

TABLE 13—*Typical consecutive measurements of insensible perspiration of a fasting marmot*

Marmot, date, weight, and time fasting	Length of period	Environmental temperature ¹	Insensible perspiration	
			Total	Per hour
R, May 26, 1936 1.15 kg. 163 days	min.	°C.	gm.	gm.
	27.4	24.3	0.40	0.88
	65.1	24.8	.65	.60
	55.4	25.4	.49	.51
	91.8	26.2	.66	.43
	57.1	27.0	.50	.53
	76.5	26.9	.63	.49
	65.6	26.3	.57	.52

¹ At end of each period.² At start of first period the temperature was 23.9° C.

A specific influence of prolonged fasting upon the insensible loss is not clearly shown, as these fasting experiments do not lend themselves for perfect comparison with the experiments on normal marmots. The insensible perspiration studies on normal marmots, the results of which are given in table 1 (page 26), dealt for the most part with measurements made at environmental temperatures of 17° or under. The profound influence of the environmental temperature upon the insensible perspiration has already been commented upon. The measurements of the insensible perspiration

during the prolonged fasts were made in late May and early June, at which time warm environmental temperatures prevailed. In the first or normal series at the cold environments the insensible perspiration averaged about 7 gm. per kilogram of body weight per 24 hours. In table 12 the values have not been expressed per kilogram. They have been computed on this basis, however, and found to be with R about 12 gm., with Y not far from 8 gm., and with S, 9 gm. All these losses are substantially higher than the losses shown in table 1. However, the effect of the prolonged fasting is complicated by the higher environmental temperature, and hence no definite conclusion can be reached as to the specific effect of prolonged fasting on the insensible loss.

RESPIRATION RATE

The marmots whose respiration rates were observed during prolonged fasting were H, I, and K, of which H was the only one that did not go into hibernation. The respiration rate per minute of H was determined from February 9 on, when the room temperature was always in the neighborhood of 1° or 2° C. The rectal temperature tended to be somewhat low, but not significantly lower than 32° except on one day, February 23, when it fell to 20.6° C. The respiration rate measured over the period from February 9 to 24 ranged from 20 to 58 per minute. A number of rates were about 25 to 30, thus confirming that 25 to 30 is approximately the minimum, non-hibernating, normal respiration rate, although in this particular case the rates were complicated by a rather prolonged fast of nearly two months. Following February 24 the marmot evidently entered into a semi-hibernating state and died shortly thereafter. There is no evidence that the fast *per se* altered significantly the respiration rate of marmot H.

The fasts of both the other marmots, I and K, were complicated by hibernation, and the counts of the respirations began only when they were practically in hibernation. Only after K came out of hibernation (marmot I having died on May 3) were any counts obtained under normal basal metabolism conditions. With K, although fasting the entire time and having lost a large proportion of its body weight (finally 72 per cent), the respiration rates varied greatly. The marmot was frequently seen to be asleep, under which condition rates of 10 per minute were recorded. On the other hand, rates as high as 70 to 80 were found on a number of days. On June 20, when the rectal temperature was 33.8°, the count was 5 per minute. The next day, when the rectal temperature was 35.0°, the count was 70 per minute. The tremendous loss in weight of K, its extreme excitability, and, to a certain extent, the variability in its rectal temperature, all tended to affect its respiration rate during fasting. It has already been pointed out (page 31) that the respiration rate of No. 5 could be doubled in one minute by excitation. A marked difference in rate was likewise noted with K, in which case the rate would be 4 on one day and 80 on the next. The extreme excitability makes it difficult to evaluate the effect of fasting *per se*.

The counts with marmot K, when it was not hibernating but had hibernated and when it had a rectal temperature averaging not far from 34° C., were frequently as low as 4, 5, or 6 per minute. These, it is true, were accompanied by a lowered rectal temperature and were noted after the fast had resulted in profound emaciation. However, as the normal basal respiration rate (see page 32) is in general 25 to 30 and the possible minimum rate is 10, it may be concluded that the prolonged fasting resulted in a lowering of the non-hibernating respiration rate. Often this was accompanied by a lowered rectal temperature and (as will be seen later—page 85) a lowered heat production.

RECTAL TEMPERATURE

With many animals a pronounced fall in rectal temperature with continued fasting has been observed. Marmots, however, because of their liberal coverings of fur and subcutaneous fat, might be considered to have sufficient protection against the cold and hence able to maintain their rectal temperatures under more severe conditions than could other animals. Against this assumption is the fact already shown that under ordinary conditions the marmot has a labile rectal temperature, that is, its temperature regulation is not good. A severe strain on this regulation is represented by the experience these marmots underwent during prolonged fasting. With marmot H the rectal temperatures were measured daily for a month while the animal was undergoing fasting. At no time did it actually enter hibernation, but in one or two instances it was sleepy. During the entire time the marmots were kept in the garage, the temperature of which ranged from 10° to 0° C. The initial rectal temperatures of H, I, and K were under 35° C. They had a tendency to vary from 33° up or down, for the most part.

With H toward the end of the fasting only one of its temperatures was under 31° C. During this time the body weight had fallen from 2.9 kg. to approximately 1.6 kg. With marmot I the initial weight was 2.5 kg. and the rectal temperature about 36°. Aside from the days when this animal was entering hibernation, the body temperatures did not, for the most part, fall below 34°, this being noted on only one occasion. With K the initial body weight was 1.6 kg. and the initial rectal temperature was 35°, but the animal shortly became sleepy and the whole series of observations was, therefore, disturbed from the fasting standpoint by the tendency to go into hibernation. On the days when K was definitely awake, the rectal temperatures remained in general about 36° C.

From table 14 (page 86), in which emphasis is laid upon the fasting metabolism measurements particularly and only the rectal temperatures incidental to those measurements are reported, it can be seen that even when the marmot had been fasting for over 100 days, its body temperature in general remained fairly high, most of these animals on the 100th day of fasting or later showing a rectal temperature of over 36° C. These particular measurements, however, are influenced by the fact that the marmots had been living

at 28° for 24 hours prior to the metabolism experiments, and the rectal temperatures had been determined after a respiration experiment in which they had been inside the chamber 2 hours.

From this study it can be concluded that when the marmot is subjected to a very cold environment of about 0° C. during prolonged fasting, the rectal temperature has a tendency to fall, showing that the heat regulation is not perfect. On the other hand, when the environmental temperature is in the zone of thermic neutrality, fasting prolonged for 150 days or more does not affect the temperature regulation and the body temperature is maintained at a normal level.

NITROGEN EXCRETION

Having established that the marmot on feed furnished by the Laboratory has an average nitrogen excretion of 0.5 gm. per kilogram of body weight per 24 hours, we hoped to study the changes in the protein metabolism as the fasting progressed. The small amounts of urine passed and the irregularity in the voiding made sharp time separations of urine expressly for comparative purposes impossible. A large number of collections were made and the samples analyzed. In spite of considerable irregularity and lack of uniformity in the amounts excreted, the general picture shown by these analyses is that while the marmots were on feed they excreted about 500 mg. of nitrogen per kilogram per 24 hours, but that when they were fasting the nitrogen excretion rapidly decreased and during the first 5 days of fasting more nearly approached 300 mg. Thereafter the excretion dropped to approximately 60 or 70 mg. per kilogram.

During hibernation, as will be shown later (page 153), there is a tremendous decrease in the nitrogen output. We were particularly interested, however, in these animals after they came out of hibernation but still continued fasting, to note whether, in spite of their initial enormous fat stores (stores that persisted even after a long winter's fast and hibernation), there would be an increase in the nitrogen output prior to death. The conventional term of "premortal" rise in nitrogen has been applied to such increases. As a matter of fact, with several of our marmots this premortal rise was found. Hence it can be stated that in spite of heavy fat deposits the protein metabolism of the marmot follows much the same course as it does with normal non-hibernating animals other than the marmot. In other words, the marmot's urinary nitrogen excretion decreases during fasting and usually undergoes a marked rise just before death from starvation.

GASEOUS METABOLISM

RESPIRATORY QUOTIENT

In the observations made on marmots during prolonged fasting the respiratory quotient was almost invariably about 0.71. In a few instances the quotient was studied on a day or two before death due to fasting. Under such conditions with marmot I there was an increase in the quotient. Thus,

on April 25 after 100 days of fasting the quotient rose to 0.76, on April 30 it was 0.77, on May 2 it was 0.80, and the animal died on May 3. These observations give clear evidence of a premortal rise in the respiratory quotient, a phenomenon that has been noted with a number of other animal species and which in the case of this marmot (as seen in table 36, page 155) was coincidental with a striking increase in the amount of nitrogen excreted.

Marmot K, which underwent a long fast with a tremendous loss in body weight, died on June 29. The respiratory quotient showed no significant rise on June 25, being 0.72, but on June 29 it rose to 0.83, evidence of a premortal rise in quotient. Marmot R, which fasted 166 days, had a respiratory quotient on May 27 of 0.77 and died on May 29. This premortal rise in quotient was invariably found in those cases where the measurements were made within three days of death.

HEAT PRODUCTION AT THERMIC NEUTRALITY

An ideal study in which the metabolism of one animal throughout an entire period of uncomplicated fasting would be measured with reasonable frequency could not be carried out, owing to the fact that most of the marmots had a tendency to hibernate. Unfortunately the one marmot with which a long series of measurements were secured on body weight and other factors, during prolonged fasting least complicated by the factor of hibernation, namely H, was not studied in the respiration chamber. However, marmots K and I along with L, R, and Y were measured on a number of occasions toward the end of their long fasts, and the metabolism data obtained are recorded in table 14. It is of special interest to note to what extent prolonged fasting affects, if at all, the metabolism under the previously stipulated condition of thermic neutrality. With these five marmots this condition was strictly met.

Although changes in body weight can not be expected to alter materially other body functions, such as heart rate and respiration rate, enough is known with regard to the effect of the size of these animals to recognize that changes in weight must be taken into consideration in comparisons of metabolism measurements where appreciable changes in weight are observed. The changes in the body weights of these fasting marmots were of a significant order. Again, therefore, we encounter the old problem of the comparison of the metabolism of the same animal at different body weights, the losses in weight being induced by fasting. Should the comparison be made on the basis of the heat production per kilogram of body weight or per $10w^{\frac{1}{2}}$? In table 14 the data are reported on three bases, as the total heat production for the day of measurement, the heat production per kilogram, and the heat production per $10w^{\frac{1}{2}}$. There are likewise added to this table for comparative purposes the basal values (italicized) for four of the five animals. Unfortunately marmot L was not measured in the basal condition.

A word of caution is necessary with regard to these so-called basal values. As has already been pointed out, the basal metabolism of the marmot can not be represented by a single value, for there is considerable lability even

in the normal, non-hibernating, basal metabolism. Nevertheless an average basal value will be of help to determine whether there are any major changes in metabolism induced by prolonged fasting. The comparison of the metabolism data for these animals on different days of fasting with their basal

TABLE 14—*Metabolism of marmots during prolonged fasting*

Marmot and date	Body weight kg.	Days fasting	Temperature		Minimum heat production per 24 hours		
			Environ- mental	Rectal	Total	Per kg.	Per 10w ²
Marmot I							
<i>Basal</i>							
April 11, 1934	2.41	1	28	38.3	82.3	34.1	461
" 16, "	1.08	86	29.0	36.3	52.5	48.6	499
" 25, "	1.05	91	28.5	36.8	57.2	54.4	554
" 30, "	.90	100	28.0	34.9	36.1	40.1	387
May 2, "	.83	105	28.0	36.8	40.8	49.2	464
	.73	107	28.3	36.5	29.6	40.6	364
Marmot K							
<i>Basal</i>							
May 7, 1934	1.60	1	28	36.7	61.2	38.3	443
" 15, "	.85	114	28.6	38.0	36.7	43.2	408
" 18, "	.79	122	28.1		36.3	45.8	424
" 21, "	.76	125	27.5		29.9	39.5	360
" 25, "	.74	128	27.9	37.5	31.0	41.9	378
" 29, "	.71	132	28.0	38.0	31.9	44.9	402
June 1, "	.68	136	28.5		27.6	40.8	358
" 4, "	.66	139	27.6		25.5	38.7	337
" 8, "	.63	142	27.9	36.8	22.1	35.1	302
" 11, "	.60	146	28.4	35.1	19.2	32.1	271
" 15, "	.58	149	28.8	37.4	21.2	36.6	322
" 18, "	.54	153	27.9	35.3	20.8	38.5	313
" 22, "	.52	156	29.7	35.2	21.0	40.4	325
" 25, "	.51	160	29.4	37.8	22.1	43.3	348
" 29, "	.49	163	27.5	34.2	16.2	33.0	255
	.46	167	27.4		6.9	14.9	116
Marmot L							
Nov. 22, 1934	1.96	8	28.0	38.6	70.7	36.1	452
" 26, "	1.84	12	28.0	38.1	66.3	36.0	445
" 28, "	1.84	14	28.8	38.9	64.9	35.3	432
" 30, "	1.79	16	27.8	37.9	53.2	29.7	361
May 1, 1935	1.28	168	27.5		56.3	44.1	479
Marmot R							
<i>Basal</i>							
May 27, 1936	2.71	1	28	34.3	73.5	27.1	388
	1.06	164	28.0	35.3	39.6	37.4	381
Marmot Y							
<i>Basal</i>							
May 27, 1936	2.32	1	28	36.6	62.0	26.7	347
" 29, "	1.17	83	28.0		55.7	47.6	502
" 1, "	1.15	85	28.0	37.4	49.4	42.9	450
June 1, "	1.12	88	28.0	34.9	43.1	38.5	399
" 16, "	.86	103	28.0	36.4	45.8	53.3	507

values is not particularly ideal, as this comparison must of necessity rest upon measurements made on one day only. It is impracticable to have a large number of long fasts on the same animal and then repeat, for example, measurements on the 70th day of fasting. In other words, whatever measure-

ment is made on the 70th day is the only one to represent that stage of fasting. This one day's measurement, however, is compared with a basal value that is frequently the average of two, three, or four days' measurements. Under these conditions the single day's measurement during fasting must be accepted with whatever fortuitous alterations in activity and nervousness of the animal existed on that particular day. Every effort has been made to select minimum periods and to rule out periods of activity, but these figures for the individual fasting days were not selected on the same basis as the basal values.

The downward trend of the body weight, the course of the respiratory quotient with the appearance not infrequently of a premortal, high respiratory quotient, and the rectal temperature during prolonged fasting have already been commented upon. The basal metabolism of each of these marmots (except L) has also already been discussed. There is reasonable uniformity in the basal metabolism of these marmots used in the fasting study; that is, marmot I had a basal heat production of 461 calories per $10w^{\frac{1}{2}}$, K of 443 calories, and R and Y lower values of 388 and 347 calories.

With I the heat production per $10w^{\frac{1}{2}}$ was reasonably uniform throughout the entire fast up to the 107th day. The variability was, however, from 554 calories on the 91st day to 364 calories on the 107th day. On this last day (see page 84) the marmot had a relatively high respiratory quotient, undoubtedly evidence of a premortal rise in the protein consumption. This, however, did not increase the heat production. The total heat production of marmot I, irrespective of body weight, changed from a basal level of 82.3 calories to a minimum of 29.6 calories on the 107th day of fasting. As the animal lost 71 per cent of its body weight during the fast, this accounts in large part for the tremendous decrease in total heat production.

Marmot K had a basal heat production of 443 calories per $10w^{\frac{1}{2}}$. On the 114th day of fasting its heat production was 408 calories and on the 122nd day, 424 calories. There was a pronounced decrease on the 125th day, and thereafter the metabolism remained essentially under 400 calories. In fact, the values from the 142nd day to the 163rd day were much nearer the 300 calorie level. On the 167th day, the day on which the animal died, there was a tremendous decrease to 116 calories. The total heat production, which at the basal level was 61.2 calories, decreased until in the last series of experiments it was down to not far from 20 calories, except on the very last day when the marmot was moribund.

Marmot L was not measured in the basal condition. On the 8th day after it was received, during which time, however, it had taken no food, the first metabolism measurement was made. On this day the rectal temperature of this animal was high, 38.6° C. The respiratory quotient, however, was that of fat, 0.72, and the heat production per $10w^{\frac{1}{2}}$ was 452 calories. On the 12th and the 14th days of fasting there was no significant alteration of the metabolism. On the 16th day the heat production dropped to 361 calories. There was then a hiatus in measurements until the next spring, when marmot L

had been without food for 168 days, during which it had hibernated a good deal of the time. The body weight had fallen to 1.28 kg., the rectal temperature was not taken, and the respiratory quotient was a trifle high, 0.75. Most important, however, is the fact that the total heat production on the 168th day had fallen to 56.3 calories as compared with an initial basal level of 70.7 calories, and the heat production per $10w^{\frac{3}{4}}$ (*i.e.*, 479 calories) had returned to its initial level.

The basal metabolism of marmot R was 388 calories per $10w^{\frac{3}{4}}$ and on the 164th day of fasting it was 381 calories. The total heat production fell from 73.5 to 39.6 calories. Hence, although there was no change in the heat production per $10w^{\frac{3}{4}}$, there was a decrease of 46 per cent in the total heat production on the 164th day of fasting.

The final series was made with marmot Y in May and June, 1936. The basal metabolism was at the low level of 347 calories per $10w^{\frac{3}{4}}$. The heat production on the 83rd to the 103rd day of fasting ranged from 399 to 507 calories and averaged 465 calories, or notably higher than the basal level of 347 calories. The loss in body weight (69 per cent) was great, and yet the *total* heat production varied only from 62 to 43 calories or only about 30 per cent, all measurements being made at 28° C .

METABOLIC REACTION TO COLD

The extremely narrow zone of thermic neutrality, almost an individual temperature ($28^{\circ}\text{ C}.$), noted with the non-hibernating marmot, led to a study of the extent to which prolonged fasting would affect the heat production when the marmots were subjected to severe cold. Two of our animals, K and L, were exposed to marked changes in environmental temperature on several occasions and a third marmot, B, had a long series of measurements made only at 22° C . This animal can, therefore, be compared with other animals at 28° but not with itself at 28° C .

With marmot K (see table 15) the chamber temperature was suddenly changed from 28° to approximately 16° C . It will be recalled that this treatment, when applied to the normal non-hibernating marmots, resulted in a greatly increased metabolism. This sudden change in temperature was not made with K until it had been fasting 122 days. It likewise was made on the 125th and 139th days of fasting. At these stages of prolonged fasting the marmot did not react to sudden changes in environmental temperature. This is a most extraordinary situation and impossible at present to interpret. Although the rectal temperature was occasionally low, yet on May 15 at the end of the experiment at the cold environment it was 36.1° C .

With marmot L experiments were made on four different days with sudden changes in temperature. At 28° the heat production was reasonably uniform on each day at not far from 430 calories. On the 12th, 14th, and 16th days of fasting there were severe changes in environmental temperature from 28° to 16° C . On these days the metabolic level at 16° was 763, 504, and 451 calories, respectively. When this marmot five months later was subjected

to 16°, then to 28°, and then to 8°, the reaction to environmental temperature was pronounced. At 28° the metabolism was 479 calories, much the same as it was throughout the entire fast at 28° C. At 16° it was 915 calories and at 8°, 1115 calories, these being essentially the averages observed in the study of the normal marmots at low temperatures.

Because with marmot L the reaction to a low environmental temperature after 167 days of fasting was substantially the same as noted with the normal animals, the absence of reaction to environmental temperature shown by marmot K is most challenging. The values for K can not be attributed to a

TABLE 15—*Reaction of the heat production of the marmot to low environmental temperatures during prolonged fasting*

Marmot and date	Body weight	Days fasting	Environmental temperature	Heat production per $10w^{\frac{2}{3}}$ per 24 hours
	kg.		°C.	cal.
1934 K May 15	0.79	122	28 16	441 473
May 18	.76	125	28 16	374 407
June 1	.66	139	28 16	345 348
L Nov. 26	1.84	12	28 16	445 763
Nov. 28	1.84	14	28 16	432 504
Nov. 30	1.79	16	28 16	361 451
May 1 ¹	1.28	167	16 28 8	915 479 1115

¹ 1935.

moribund condition, for the animal still continued living and was subjected to metabolism measurements for one month longer.

Marmot B had food withdrawn on June 27. Consequently one could not assume that there was any likelihood of its going into hibernation at this time of year. The environmental temperature during the measurements was held at 22° C. This animal fasted 96 days, and as a temperature of 22° is below that assumed to be thermic neutrality for the non-hibernating marmot, one would expect B to have a somewhat high heat production in spite of the fasting itself. The temperature at which B lived during the fast varied according to the outdoor temperature and ultimately was higher than 22° for not a little part of this time. The first measurement of 554 calories per $10w^{\frac{2}{3}}$ represents, judging from our experience with other animals at a temperature 6° below the critical temperature, a low metabolism. One would

have expected the reaction to the temperature to result in a higher heat production than this. However, the metabolic level was maintained between essentially 550 and 600 calories for the first 8 days of fasting, with insignificant changes in rectal temperature varying only from 36.7° to 37.1° C. (See table 16.) Rather suddenly on July 7, the 10th day of fasting, the heat production decreased to 494 calories and remained at about this level through July 27. On August 5, the 39th day of fasting, there was another sudden drop of 100 calories in the heat production per $10w^{\frac{3}{2}}$, and a second measurement on August 11 confirmed this. On both of these days the rectal temperature was relatively low. On August 19, September 16, and September 23 the heat production was greatly increased, and the rectal temperature was slightly increased from the 34°-level observed on August 5 and August 11. Finally,

TABLE 16—*Metabolism of marmot B during prolonged fasting¹*

Date	Body weight kg.	Days fasting	Rectal temperature ² °C.	Minimum heat production per 24 hours	
				Per kg.	Per $10w^{\frac{3}{2}}$
1932					
June 28	3.06	1	37.0	38.2	554
" 29	2.98	2	37.0	40.8	588
" 30	3.03	3	36.7	37.7	544
July 1	2.93	4	37.1	41.0	586
" 5	2.83	8	37.0	43.6	617
" 7	2.86	10	37.5	34.8	494
" 15	2.67	18	36.6	34.3	476
" 21	2.60	24	37.0	30.5	419
" 27	2.52	30	35.6	35.4	483
Aug. 5	2.41	39	34.5	28.4	380
" 11	2.35	45	33.7	27.3	364
" 19	2.30	53	36.0	35.6	469
Sept. 16	2.07	81	36.1	40.3	515
" 23	2.04	88	36.5	43.8	564
" 30	1.96	95	30.3	30.9	388

¹ Environmental temperature, 22° C.

² End of experiment, unless otherwise stated.

³ At beginning of experiment.

on September 30, when there was a tremendous drop in the rectal temperature to 30.3° C., the heat production decreased to 388 calories, although this was not absolutely the lowest level, for on two earlier days values of 380 and 364 calories were noted.

From the standpoint of reaction to environmental temperature these observations on marmot B do not show a greatly increased heat production at 22° over the level that would be expected during a fairly long fast at 28°. It would, therefore, appear as if the data for marmot B tend to support the finding with animal K, namely, that the reaction to environmental temperature is not so profound during fasting as it is prior to fasting. The single anomalous situation is, however, that in the very first few days of fasting marmot B's average metabolism of 560 calories at an environmental temperature of 22° was lower than one would expect from the study with the larger normal series. In our basal table 6 (page 61) the selected average

minimum value for B at the critical temperature of 28°, that is, 500 calories, is high compared with the values for the other marmots. Hence the lowering of the temperature from 28° to 22° C. in the case of marmot B produced an average increase in heat production of only approximately 60 calories, which is a much smaller increase than one would expect, judging from the experiments with other normal marmots.

General conclusions with regard to the effect of prolonged fasting on the metabolism

Considering only those measurements made at thermic neutrality and recognizing that at least one of the marmots presents a very singular condition of not reacting to great changes in environmental temperature after prolonged fasting, we may draw conclusions as to the effect of prolonged fasting upon the heat production of the marmot. In general, according to the averages in table 14, the total heat production decreases as the fasting progresses and is roughly proportional to the loss in body weight. With many animals when fasting it is considered that the so-called "surface-area law" does not hold and that there is a decrease in the heat production per square meter of body surface as the weight decreases, but it is argued that the heat production per unit of body weight has a tendency to remain essentially constant. For these fasting marmots the heat production has been reported on both bases. The general picture is that the fasting heat production per kilogram of body weight varies with the animal. With marmot I it tends to increase. With K it remains singularly constant. With marmot L it decreases slightly, and with R and Y definitely increases. Examination of the body weights of these animals, however, indicates that those marmots whose heat production per kilogram increased as the fast progressed were the largest animals. Hence, although losing weight, they had retained a larger proportion of their heat-producing mechanisms. Of the two smaller animals, K and L, the first had the greatest loss in weight of any of the marmots, 72 per cent. Although marmot I had experienced the same percentage loss by the end of its fast, it had a body weight nearly twice that of K at death. It, therefore, appears as if with K there was not simply a loss of heat-producing mechanism but a complete lowering of the body tonus resulting from the unusually long fast, which was 60 days longer than that of marmot I. Similarly L, which had the longest fast of all, 173 days, showed a decrease in the heat production per kilogram.

From the standpoint of the heat production per $10w^{\frac{3}{4}}$ the metabolism was less variable from day to day throughout the fast than was the heat production per kilogram. This is true with all the animals, although there were striking exceptions on the last two days of observation with K. So far as the evidence goes, if one is inclined to accept unqualifiedly the surface-area law, these experiments must support the contention that in prolonged fasting the heat production per unit of surface area is constant with different animals of the same species. To what extent this is associated with heat lost from

the surface area and to what extent it is a result of factors working upon the metabolism of these animals still remains unsettled.

Thus from the standpoint of the total heat production the effect of prolonged fasting is, as one would expect, in general a decrease in heat production as the body weight decreases. On the basis of the heat production per kilogram the larger marmots show an increase and the smaller marmots a decrease in metabolism. On the basis of the heat production per $10w^{\frac{3}{4}}$ the general picture is a moderate degree of uniformity, save for the last two days with marmot K.

With marmot B, measured at 22° C., the picture is by no means so clear. There were wide fluctuations up and down, both in the heat production per kilogram and that per $10w^{\frac{3}{4}}$, and there was no pronounced correlation between these fluctuations and the length of the fast. Although the reaction of B to a lower environmental temperature was not so great as one would expect with a normal marmot, nevertheless it is possible that this reaction complicated somewhat the normal course of the heat production as influenced by fasting alone.

The general deduction, therefore, is that the heat production per $10w^{\frac{3}{4}}$ is, for the most part, independent of the size of the animal as well as the length of the fast, a generalization indicating a most singular heat production of an animal that has undergone tremendous changes in weight due to deprivation of food. It is only when death is approaching and the animal is in the moribund condition that general deviations in the average heat production are to be found. Therefore, prolonged fasting, even to the extent carried out with these marmots, may be said to be without great effect upon the heat production either per kilogram or per $10w^{\frac{3}{4}}$, for values that range well within the normal limits were found on these two bases throughout the entire fasting periods. This picture is of itself in striking contrast to that found with most other animals during prolonged fasting, which show not only a decrease in the total heat production as the body weight decreases but likewise an almost invariable tendency for the heat production per kilogram and per $10w^{\frac{3}{4}}$ to decrease.

WATER VAPORIZED FROM THE LUNGS AND SKIN

Because the vaporization of water is a path of heat elimination from the body, a study of this function under prolonged fasting was made. The vaporization of water is of chief significance in relation to the total heat loss. The results, therefore, will be compared on the basis of the percentage of heat lost in water vapor. Marmot B, used in this study, was measured at 22° , unfortunately not the critical temperature for the non-hibernating marmot, but as all its experiments were made at this temperature the course of the percentage losses in heat by vaporization of water will give an accurate picture of the effect of the prolonged fast. The percentages on the 10th, 18th, and 24th days of fasting were 9, 15, and 15, respectively, averaging 13 per cent. On the 30th day the level was much higher, 28 per cent. How-

ever, on the 39th and 45th days percentages of 13 and 21 were found. Thereafter the heat losses through the medium of water vapor were all remarkably constant, being 12, 11, 11, and 14 per cent on the 53rd, 81st, 88th, and 95th days of fasting. Although there were some variations, the progressive stages of fasting did not significantly alter the percentage of heat lost by the vaporization of water. For normal non-hibernating marmots the percentage of heat lost through the path of water vapor was 20 per cent at 28° and 7.8 per cent at 16° C. Thus the losses of about 12 per cent toward the end of the prolonged fasting at 22° are in line with these values, from which it is concluded that there is no significant alteration in the proportion of total heat lost by vaporization of water ascribable to prolonged fasting.

BODY COMPOSITION AFTER PROLONGED FASTING

In these studies over extended periods when no food was consumed, measurable amounts of nitrogen were excreted, as already shown. It is, therefore, essential to consider the changes that occurred in the body composition over this

TABLE 17—*Body composition of marmots after death from starvation*

Marmot	Days fasting	Initial weight gm.	Dead weight gm.	Per cent moisture in dead weight	Dry dead weight			Bones		Nitrogen ¹		Fat ¹		Ash ¹	
								Per cent of							
					Flesh, skin, fur gm.	Bones gm.	Total gm.	Initial weight gm.	Dry dead weight gm.	Grams	Per cent ²	Grams	Per cent ²	Grams	Per cent ²
H	48	2920	1219	70	256	107	363	3.7	29.5	34.9	9.6	17.6	4.8	10.6	2.9
I	107	2466	725	72	132	68	200	2.8	34.0	15.4	7.2	11.8	5.9	5.7	2.9
K	167	1644	463	70	88	51	139	3.1	36.7	10.4	7.5	10.7	8.7	4.5	3.2

¹ Determined in flesh, skin, and fur.

² On basis of dry matter in dead weight.

period. As it is obviously impossible to obtain data on the exact composition of the body prior to fasting and even a large mass of data on non-fasting animals would not be an accurate baseline for individual animals, certain refinements in this study of body composition were not observed. Specifically, the bones were not analyzed, although it is realized that some drafts upon the nitrogen, fat, and mineral salts in the bones take place during fasting. In the case of the three marmots, H, I, and K, which died as a result of starvation, analyses were made of their boneless carcasses. (See table 17.) At death marmot I weighed about 1½ times as much as K, and H nearly 3 times as much as K. Comparison of the total dry weights at death also shows these same ratios, which means that there was extraordinary uniformity in the percentage of water content, *i.e.*, 71 per cent. The weight of bones is a more uniform percentage of the initial weight than of the dry dead weight, the range in percentages being only from 2.8 to 3.7 in the former case but from 29.5 to 36.7 in the latter case. The weights of total nitrogen exclusive of that in the bones were in the same ratio to each other

as the dry weights and again nearly uniform values on the percentage basis were found, averaging 8.1 per cent.

For marmot I we can make a rough estimate of the minimum quantity of body fat that was consumed during the 107-day fast, using the minimum heat production measured each week and assuming the lowest level during hibernation. The total heat production during this time would be approximately 4000 calories, of which 360 calories would be supplied by protein, according to the urinary nitrogen excretion during the fast. This leaves 3640 calories to be supplied by fat, and as the caloric value of one gram of fat is 9.5, 383 gm. of fat would be required. In view of the fact that this is a minimum estimate, the marmot must have had a large quantity of body fat at the start of its prolonged fast.

Marmot H died early, after only 48 days of fasting. It still had 17.6 gm. of fat available in the body. Comment has already been made of the fact that H had an increased nitrogen output at the end of its fast, which has

TABLE 18—*Body composition of normal marmots*

Marmot	Dead weight kg.	Per cent moisture	Dry weight gm.	Bones		Fat	
				Dry weight gm.	Per cent of dead weight	Weight gm.	Per cent of dry matter
7	3.21	41.7	1870	211	6.6		
8	1.54	34.5	1008	130	8.4		
18	1.30	24.9	980	283	11.9		
20	2.57	59.2	1046	193	7.5	491	46.9
21	2.64	43.0	1512	155	5.9	1732	48.4

¹ Some lost.

been interpreted as being a premortal rise. A premortal rise in both the respiratory quotient and the nitrogen excretion was noted with marmot I and likewise with K, although these animals had 11.8 gm. and 10.7 gm. of fat, respectively, in their dead bodies. Thus marmot K, with the smallest body weight at the start, lived the longest time and yet had the largest percentage of fat at death. The percentages of ash were uniform.

For comparison with the marmots that died from starvation, the moisture contents of the bodies of five normal marmots were determined, and in two of these cases the fat was extracted. (See table 18.) The moisture contents had a fairly wide range, from 25 to 59 per cent, and averaged 41 per cent. For the marmots that died from starvation the percentages of water were uniform at 71 per cent. Thus the fasting marmot's water content is relatively high, being almost twice that of the normal marmot. This increase in the percentage of water as a result of fasting has been frequently noted with other animals. All the normal marmots had larger weights of bones than did the animals that starved to death. The weight of dry bones in the normal marmot is 8.1 per cent of the dead weight and in the case of the starved marmot the dry bones are 3.2 per cent of the initial weight. Thus the bones of the starved marmot represent a smaller proportion of the body,

which suggests that rather heavy drafts upon bone substance are made during prolonged fasting.

Analyses of two of the normal marmots gave 48.4 and 46.9 per cent of dry matter as fat. The drafts upon body fat by marmot I during its 107-day fast have been roughly calculated to amount to 383 gm. This value is obviously too low, as minimum values for the caloric output were used in the calculations. Had the activity of the animal been taken into account in the calculation, this minimum value of 383 gm. would have been considerably increased, probably approximating that found in the bodies of the normal marmots.

From these analyses it is clear that fasting, prolonged to death, results in marked increases in the percentage of moisture in the marmot's body and almost total depletion of the body fat.

GENERAL CONCLUSIONS AS TO THE EFFECT OF PROLONGED FASTING

The non-hibernating marmot in its ordinary life has a rectal temperature essentially that of other mammals. Its basal metabolism is considerably lower than that of other mammals, to be sure, but certainly from all external appearances one can consider the non-hibernating marmot a warm-blooded animal. Judged on the basis of the further criteria of its resistance and reactions to prolonged fasting, this animal responds to fasting as do other mammals, so far as its rectal temperature, its respiration rate, and its losses in body weight are concerned, thus indicating that it is closely allied to warm-blooded animals in general. In two particulars the marmot shows a different effect of prolonged fasting from that of other warm-blooded animals. The marmot when subjected to prolonged fasting with consequent enormous losses in body weight has a heat production far more regular per $10w^{\frac{1}{3}}$ (*i.e.*, the approximate surface area) than do other warm-blooded animals. With practically all other mammals the surface-area calculations do not give uniform results, but uniformity is more closely approximated by the expression of the heat production per kilogram of body weight.

In one other point the marmot at times shows a striking difference from the ordinary mammal in its reaction to prolonged fasting. Certain marmots, at least, after fasting appear to lose their power or to have a greatly diminished power to react to lowered environmental temperatures. This might, however, be considered as a distinctly advantageous factor in prolonged winter sleep. No adequate explanation for this phenomenon is at present available. Practically all the evidence, however, shows that the non-hibernating marmot essentially represents a normal warm-blooded animal.

PHYSIOLOGY OF THE MARMOT WHEN ENTERING HIBERNATION

GENERAL CONDITIONS PREVAILING IN TRANSITIONAL STATE

Although comparison of the marmot's metabolism when hibernating with that when it is awake and under basal conditions is of tremendous physiological importance, no observations can contribute more to an interpretation of the marmot's physiology in the hibernating state than those made regarding the various body functions of the animal as it enters hibernation and in the subsequent waking-up period. Of these two states it was far easier to study the waking-up condition. Marmots in deep hibernation could be frequently found in the Nutrition Laboratory's collection. They could then be awakened by various means and the extraordinary waking processes studied in detail. Of equal and perhaps of even more importance physiologically in interpreting the life processes during hibernation would be a study of the transitions as the animal *enters* hibernation. The waking process may be extraordinarily rapid, usually a matter of but an hour or two, but the process of entering hibernation is of an entirely different time order.

In all cases our marmots were under artificial conditions, being housed usually in cages in a garage. Hence our observations will contribute data as to the reaction of these animals only under laboratory-controlled conditions. Based upon the general knowledge that marmots hibernate in cold weather after food becomes scarce or not available and live in burrows underground at a temperature above freezing, the marmots were, for the most part, kept in the garage at a temperature of about 10° C., and the garage temperature was never allowed to go below freezing, a condition that would not normally exist in the burrow. When we were particularly interested in having the marmots go into hibernation, food was removed, and to further simulate the condition of the burrow the windows were covered so that the garage would be dark at all times. This also prevented any sunlight from entering and causing local warming of an individual animal.

General observations were made daily on the animals when it was suspected that hibernation might occur. It is quite possible that animals in the open will hibernate at a much earlier date than they did in the Nutrition Laboratory. Indeed, one marmot that was received at the Laboratory on November 14 (L) was reported as being "dug out" of hibernation.

The whole series of observations shows that the process of entering into hibernation is not rapid. It does not take place within an hour or two, and the attainment of the actual deep hibernating state is usually a matter of days. In a survey of all the marmots that hibernated and upon which daily observations were made, it was found that the animals first became lethargic and uninterested in the general conditions surrounding them, so long as they

were not in harm's way. Usually the animal's eyes were open and it was not in the characteristic curled-up or ball-shaped position of deep hibernation. Frequently the marmot would apparently be sleeping, and the noise incidental to some one's entering the garage would cause it to raise its head, or the disturbance of the observer working in the garage would produce a similar reaction.

The earliest date under laboratory care when a drowsy condition was noted was in the case of No. 25 on November 19, 1936. Another marmot, D, in an earlier series in 1932 was observed to be drowsy on November 29. These are the only two animals which under laboratory conditions showed signs of becoming lethargic prior to the month of December. However, these marmots were frequently maintained at warm temperatures during the month of November, often as high as 28° C. for basal metabolism measurements. Furthermore, as it was desired in the basal series to have the animals in as good condition as possible, they were usually fed so that the time after food when the metabolism was measured would be known with some degree of certainty. Early in December several animals were noted to be sleepy for the first time. In fact, eleven of twenty-six marmots observed specifically showed signs of stupor prior to December 16.

The process of entering hibernation may be considered to take place in steps, for the marmot returns to its original awake condition at least once after its initial drowsiness. The marmots that became sleepy usually maintained this condition for about one day at the start. This was not always the case, but over half the animals followed this routine. The actual period of initial lethargy varied from a few hours to four days, after which the marmots returned to the normal awake state. Usually the second step into the semi-hibernating state occurred from one to five days after this first drowsy period. Some marmots maintained this second drowsy state for only a day or two and thereafter the condition reappeared every two or three days. A few marmots, and only a few, apparently went definitely into hibernation within a very short time after the initial period of drowsiness. The time after the initial period until the marmots went into deep hibernation varied from three days to a month. Some animals never actually went into true, deep hibernation, although they were in a stuporous condition on several occasions.

Usually the marmots did not hibernate until after food had been removed, but in the case of No. 25 this was not true. This particular animal was living with fourteen other marmots in a metallic chamber, which was open at the top and of large dimensions, having a floor area of approximately 3 square meters. Two pans of food and a pan of clean water were usually available to the animals. Fresh food was given daily. It is possible that No. 25 either was not interested in food or perhaps could not get its share easily. Only one other marmot, No. 9, showed signs of lethargy when it had food available. Thus the availability of food seems to have been a detriment to obtaining the condition of hibernation. It has been noted

that animals in zoological parks seldom hibernate. As these animals are fed throughout the winter, it can be inferred from this standpoint that hibernation is a method of self-preservation or conservation of the body reserves when food is not available.

RECTAL TEMPERATURE

The observations just cited as to the condition of the marmot during the transition into hibernation, although made by experienced observers, are superficial and are not of more than general physiological value. In most of the measurements of the physiological functions it is necessary to handle the animal in some manner. Perhaps the only exception to this would be the counts of the respiration rate, which will be discussed later. One of the truest and most illuminating measurements is that of the rectal temperature. This unfortunately requires the insertion either of a thermo-junction or a mercury thermometer for some distance into the rectum and usually the handling of the marmot in order to hold it in a position permitting insertion of the thermometer. The insertion itself is a process that disturbs the animal, although not infrequently we were able to insert the thermometer with a minimum amount of disturbance to the animal. As the rectal temperature of the marmot in the awake or normal condition averages 36° to 37° C. and as the marmot in deep hibernation has a temperature much like that of its environment, any rectal temperature notably higher than the environmental temperature and yet significantly below 36° will indicate that the marmot is in the process either of entering or awakening from hibernation.

As previously pointed out, the process of entering hibernation is a gradual one and takes place in steps. No. 25, which on November 19 was in a drowsy condition, had a rectal temperature one-half hour after being transferred to an individual cage slightly below 29° , but this rose rapidly to 33° C. At the time of measurement the animal was semi-conscious due to the stimulation of handling, and it is very likely that it had a temperature lower than 29° when first found in lethargy. The environmental temperature of the garage on this day was 0° C.

With another marmot, D (see table 19), on November 29 at 9.07 a.m. the rectal temperature was 34.4° and at 4.25 p.m. was 22.5° C. This measurement was made with a rectal thermo-junction and was immediately confirmed by a mercurial thermometer reading. Another measurement at 4.30 p.m. with the thermo-junction showed the temperature to be 24.5° C. This rise in temperature of 2° was probably caused by the handling of the animal. On the following day, November 30, the animal's temperature had risen to 32.5° , not significantly below normal. Although, as has been stated, the process of determining the rectal temperature necessitates handling and this will cause the animal to awaken, the temperature is usually measured in a short period of time, under which conditions the animal is not able to increase its body temperature significantly. Thus these low temperatures are approximately the levels at which the marmot has been prior to the measurements,

the undesirable but necessary factor of handling being a stimulus upsetting the normal course of the body temperature. In the afternoon of December 14, marmot D had a rectal temperature of 33.1° C. On the following day, December 15, the temperature had dropped to 28.7° C. at 9.15 a.m., but 7 hours later the temperature was back near its normal level, i.e., 34.0° C. These measurements offer another indication of the steps by which the marmot adjusts its physiological functions to the stage of entering hibernation. On the afternoon of December 16 marmot D had a rectal temperature of 34.4° C. It was not measured on December 17 and 18, but on December 19 the animal is recorded as having hibernated, its temperature being 5° C. at

TABLE 19—*Rectal temperature study with marmots D, E, and F*
(15 cm. insertion in rectum)

Date	Environ-mental temperature °C.	Marmot D		Marmot E		Marmot F	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1932							
Nov. 28	4.0	34.6	34.4				
" 29	4.3	34.4	22.5			32.9	
" 30	7.3	32.5	30.6	32.1	34.5	34.7	
Dec. 1	10.0	34.2	31.4	33.0	30.8	33.1	35.4
" 2	9.8	35.4	33.5				
" 6	11.3			32.3	33.6	32.8	32.8
" 7	13.8	34.1	31.4				
" 8	10.5			32.1	33.5	33.9	35.0
" 9	8.0	32.3	32.9				
" 13	4.3	34.4	34.8	34.0	34.2	34.4	34.0
" 14	4.5	33.9	33.1	33.4	34.2		
" 15	4.3	28.7	34.0	33.3	33.4	33.8	34.0
" 16	1.8	34.0	34.4	34.0	31.2	33.3	26.1
" 19	4.5	15.4	35.2	32.7	30.6	29.8	24.2
" 20	4.3	²		31.2	23.6	34.1	27.1
" 21	5.0	6.5	13.4	35.1	29.0	32.1	28.3
" 22	6.3	20.8	12.1	34.2	26.9	28.3	23.4
" 23	6.0	6.5	15.6	23.3		21.9	21.3
" 24	6.0	8.1					

¹ Checked with mercury thermometer.

² Appeared to be going to sleep again.

³ Gradual constant rise from 9.20 a.m. to 4.26 p.m. Temperature measured every ten minutes; thermo-junction remained in place.

⁴ Thermo-junction in place overnight, but found bitten in two on December 22, and a new junction was used to make this measurement.

9.22 a.m. The animal woke up before the end of the day, but was reported as being sleepy again on December 20. No measurements were made on December 20, as it was desired not to disturb the animal. On December 21 this marmot was measured every 10 minutes, and its rectal temperature rose from an initial level of 6.5° at 9.20 a.m. to 13.4° at 4.36 p.m., there being a constant increase during the day.

With marmot E the first lowering in the rectal temperature might be considered to be on December 1. In the morning its temperature was 33°, but in the afternoon a temperature slightly below 31° was recorded. The temperatures from there on for a number of days were always above 32° and

usually more nearly in the vicinity of 33° or 34° C. The next temperature below 32° occurred on the afternoon of December 16, being 31.2° C. On December 19 the temperature was 32.7° in the morning and 30.6° in the afternoon. The following day, December 20, the morning temperature was 31.2°, showing that a level slightly below the normal was being maintained. This may be taken as an indication of the fluctuations in rectal temperature. On the afternoon of this day the temperature had fallen to 23.6°, a drop of over 7° in 7 hours. On the morning of the following day the temperature had reached the highest level for several days, 35.1°, but in the afternoon it had fallen to 29°, here again a fast drop of almost one degree per hour. On December 22 the morning temperature was high, 34.2°, and the afternoon temperature was 26.9° C. The following morning, December 23, the temperature had fallen still further, showing another lower step or dip in the process of entering hibernation.

Marmot F maintained a temperature of 32.8° and above until December 16. On this day the morning measurement established the rectal temperature at 33.3°, but in the afternoon the temperature was 26.1°C. The temperature was not measured on the next two days, but on December 19 the morning level was 29.8° and the afternoon level 24.2° C. The next two days are both characterized by a nearly normal level in the morning, averaging 33°, and a lower level in the afternoon of about 27.5°. The temperature was noted as being in general progressively lower on the next two days, namely, 28.3° on December 22 in the morning, 23.4° in the afternoon, 21.9° the next morning, and 21.3° or half a degree lower in the afternoon.

The environmental temperature for this series of measurements on marmots D, E, and F ranged from 0° to 15.5°, but for the most part averaged not far from 6°C.

Thus it is shown that the initial stage of entering hibernation occurs by dips. The marmot never goes directly into deep hibernation the first time it becomes drowsy but usually it returns to approximately its non-hibernating level of living. These dips may or may not occur several times. After the initial lowering in temperature, which amounts to only a few degrees, the marmot is capable of undergoing more rapid changes and may go into deep hibernation directly from a higher body temperature level. This is illustrated in table 20 by marmot I. On February 17 this animal had a rectal temperature of 36.2° and two days later of 16.8°; on February 21 its temperature was 33.5° and two days later 6.9° C. In these two cases there were two days elapsing between the measurements, but on March 28 the same animal had a temperature of 35° and the following day the temperature was 10.8° C. Marmot K also underwent rapid transitions, on March 23 its temperature being 35.4° and on March 24, 8.4°. Again on April 12 its temperature was 37.8° and the following day it was 14.2°. In these cases a decrease of 15° or more occurred in approximately 24 hours.

It has also been pointed out (table 19) that marmot D on two occasions in a period of 2 days underwent a decrease in rectal temperature from a level

of approximately 34° or 35° down to 5° or 6°, namely, on December 16 and 19 and on December 19 and 21. On December 21 the temperature in the

TABLE 20—*Comparison of rectal temperatures and respiration rates of marmots I and K*

Date	Room temperature	Marmot I		Marmot K	
		Rectal temperature	Respiration rate per minute	Rectal temperature	Respiration rate per minute
1934	°C.	°C.		°C.	
Feb. 9	-1.0	18.4	12	11.3	14
" 10	-1.0	24.8	36	11.8	17
" 12	1.0	11.0	13	6.8	10
" 13	4.5	15.4	13	8.5	6
" 14	1.5	15.3	11	6.9	7
" 15	0.0	35.5	17	13.3	22
" 16	0.0	36.4	29	11.5	17
" 17	1.0	36.2	58	6.4	8
" 19	2.0	16.8	7	6.1	7
" 20	5.0	11.6	8	12.7	10
" 21	4.0	33.5	5	6.8	9
" 23	6.0	6.9	4	6.9	12
" 24	2.5	15.4	10	9.7	8
March 22	6.9	10.2	0.4	11.1	3
" 23	3.0	8.0	6	35.4	48
" 24	2.8	5.8	5	8.4	8
" 26	9.3	7.1	0.7	8.4	0.9
" 27	13	10.6	3	10.7	0.7
" 28	28	35.0	50		
" 29	11	10.8	0.4	15.7	0.2
" 30	14	11.4	1	12.1	1
" 31	12	11.5	0.5	11.9	1
April 2	14	12.0	0.5	12.1	0.7
" 3	16	15.5	1	17.0	3
" 4	18	16.4	0.6	18.1	3
" 5	13	14.6	31	25.6	0.7
" 6	14	13.6	0.5	15.5	5
" 7	13	15.8	14	13.8	2
" 9	16	14.4	0.4	33.1	2
" 11	14	14.3	6	37.2	14
" 12	12	14.4	1	37.8	23
" 13	10	11.0	0.6	14.2	11
" 14	12	10.7	0.2	11.3	18
" 16	27	32.5	17	37.8	100
" 17	28	37.1	71	38.0	167
" 18	28	28.0	1		
" 20	23	24.0	2	36.5	100
" 21	30	36.8	50	37.4	111
" 23	29	34.9	6	36.5	100
" 24	27	37.0	100	37.1	125
" 25	28	34.9	11	37.5	100
" 26	27	34.4	7	37.4	143
" 27	30	36.8	18	37.5	100
" 28	28	31.7	6	36.5	100
" 30	29	33.7	6	36.7	99
May 1	29	36.5	9	36.7	90
" 2	30	36.5	5	36.4	50
" 3	29			37.6	100
" 4	29			37.1	90
" 5	28			37.1	90

morning was 6.5°. During the day the thermo-junction was left in place and the temperature measured every 10 minutes. Each record was slightly

higher than the one before, and the steady rise is clearly indicative of the change from a hibernating condition toward a waking condition. Further evidence that this animal woke up was the fact that the thermo-junction, which had been left in place at 4.36 p.m. on December 21, was found destroyed on December 22, thus confirming the belief that the marmot had regained consciousness during the night. As will be noted subsequently (page 183) when the transition from hibernation to the awake condition is discussed, this process of waking seldom stops before the marmot has fully regained its normal body temperature. It is, therefore, reasonable to assume that sometime after 4.30 p.m. on December 21 marmot D attained a temperature of about 34° or 35°C. The temperature measured on December 22 at 9.11 a.m. had fallen to 20.8° and in the afternoon it fell still further to 12.1° C., a drop of 8.5° in 7 hours.

Perhaps the most striking fall in temperature of a marmot entering hibernation was shown by the measurements on marmot D on January 19, 1933, the details of which are as follows:

Time	Rectal temperature °C.
9.30 a.m.	36.0
10.30 "	35.3
11.30 "	35.3
12.30 p.m.	36.2
1.30 "	34.0
2.30 "	34.0
3.30 "	31.2
4.30 "	27.7
5.30 "	25.1

On this—a rare—occasion it was possible to insert the thermo-junction in the rectum of the marmot when it was awake and leave it without the animal's destroying it. The environmental temperature during the day averaged 8°C. The rectal temperature from 9.30 a.m. to 12.30 p.m. was maintained at 35.3° or above and at 12.30 p.m. was 36.2°. An hour later it had dropped two degrees to 34°. During the next hour it did not drop, but in the successive hours it dropped 2.8°, 3.5°, and 2.6°, respectively. Thus from 12.30 p.m. to 5.30 p.m. or in 5 hours the temperature had dropped from 36.2° to 25.1° or 11° C. This would average two degrees per hour, which shows the extraordinary rate at which the temperatures of these animals can change from the warm level to the cold level. The next day, January 20, marmot D had a rectal temperature of approximately 10° C.

There are, therefore, two ways in which the marmot goes from the warm body temperature to the cold body temperature. In the first or initial step the lowering in temperature amounts to only a few degrees, and the marmot usually warms up again. This process is relatively slow, but after the marmot has once been in the hibernating state it is possible for its temperature to drop from the warm-blooded level to the cold-blooded level in a few hours. In fact, in one instance a marmot's temperature dropped 11° C. in five hours, or essentially two degrees per hour.

BODY WEIGHT CHANGES

In view of the profound difference in the metabolism of the marmot when awake and when in deep hibernation (a difference that, as will be seen later, must occur when there is a marked lowering in body temperature, for the body temperature is a resultant of heat production and heat loss), one would expect in the transition periods correspondingly intense changes in body weight, respiration rate, water-vapor output, and heat production. As these changes, particularly when the marmot is entering hibernation, are relatively slow and occur in steps, theoretically it should be possible to note the gradations in these changes. But many of these observations necessitate contact with the animal, which tends to stimulate it to awaken. Inasmuch as the insensible weight loss of the non-hibernating marmot during the early hours of fasting corresponds at 12° to about 6 gm. per kilogram per 24 hours and that of the hibernating marmot amounts to but one-thirtieth of this (see pages 26 and 120), there should be, theoretically, a transition in the rate of weight loss during the change from the awake condition to hibernation.

It will be recalled that the body weight curve for marmot H in figure 6 represents the effect of prolonged fasting practically uncomplicated by hibernation and that throughout the entire period of study the decrease in weight is represented by a fairly straight line. With marmot I there was a correspondingly rapid decrease in weight until the animal began to show signs of sleep, approaching hibernation, a state indicated in figure 6 by the hollow circles. There was certainly no definitely outlined change in the rate of fall with either marmot I or K until hibernation took place. From there on the weight loss was at a much slower rate. Evidently the small changes in body weight and the time involved are not such as to give a clear picture of the gradation of loss in the transition period from being awake to hibernation.

Throughout the entire study we were interested in securing, if possible, criteria indicating that the marmot was entering hibernation, preferably criteria that would not necessitate contact with or handling of the animal. It is evident that changes in the rate of weight loss can hardly be relied upon, for even the observations of these changes made it necessary to suspend the marmot on the balance, which meant a certain amount of disturbance to the animal.

INSENSIBLE PERSPIRATION

The insensible perspiration of an animal obviously going into hibernation or at least at a semi-steady state en route to hibernation, with a low rectal temperature, is shown by the results obtained on marmot F (weight, 1.3 kg.) on January 4, 1933. The rectal temperature throughout the day averaged 23° C. The animal had urinated early in the morning, and although the balance and the cage in which it was suspended were carefully dried, it is more than likely that some moisture adhered to the animal's fur, for the insensible loss in the first hour was much larger than that of a marmot when awake. From 10.32 a.m. to 3.22 p.m. or for 5 hours, the loss was 1.99 gm. per kilogram per 24 hours. During the next hour the loss was so small as

to be not readily weighable. This loss of practically 2 gm. per kilogram per 24 hours at a rectal temperature of 23° is only about one-fourth or one-fifth of that of the average marmot fully awake, but is measurably higher than that of the animal in deep torpor which is 0.2 gm. per kilogram per 24 hours. (See page 120.) Hence this criterion of the insensible loss is in harmony with the idea that the animal was in partial torpor.

RESPIRATION RATE

Many of our marmots were watched closely from day to day and, indeed, from hour to hour in the attempt to note the precise conditions most favorable to induce the marmot to enter hibernation when relatively unmolested, and to note just what factors are incidental to its going into hibernation. Superficial observations would be of relatively little value. We were particularly interested in securing physiological measurements, the simplest of all perhaps being the respiration rate which could be observed without disturbing the animal. Records of the heart rate were not feasible in most cases, and it was always disturbing to take the rectal temperature, because the mere introduction of the thermometer acted as a stimulus and either retarded the entering hibernation or awakened the marmot completely, thus defeating our ends of securing a large number of observations during hibernation. It was hoped from these attempts to study the marmot when going into hibernation, uncomplicated by handling or other disturbance, if possible, to throw light on what circumstances favor the hibernation of the marmot, what are the causes of hibernation, and what are the various physiological steps involved.

In the early stages of going into hibernation, however, when the animals were still active, even the approach of the observer to take the respiration rate many times stimulated the animal, delaying, if not resulting in the complete cessation of, the entering into hibernation. Furthermore, the effect upon the respiration rate of the extreme sensitivity of the marmot was such, even in normal basal metabolism measurements, that the use of the respiration rate as an index of approaching hibernation was by no means certain. Although many marmots had rates at times, when awake, as high as 140, our conclusion was that the average quiescent, non-hibernating basal rate would be not far from 25 to 30 per minute. Rates notably under this were usually considered as indicative that the marmot was entering hibernation, if it had not absolutely arrived there.

The respiration rates of a large number of marmots between the waking condition and the hibernating stage were noted, but without a more definite index of the condition of the animal such records are not quantitative at best and are not of any great value. The extreme sensitivity of the marmot to any stimulus affects the respiration rate momentarily and pronouncedly, although often after such stimulus the respiration rate returns rapidly to the lower level. However, such stimulus makes respiration rates obtained just prior to the taking of the rectal temperature somewhat uncertain. A long series was secured with marmots I and K from February 9, 1934, to well into

the spring when the animals began to wake up. Disregarding for the moment those weeks when the marmots were awake and beginning our analysis of the data only at the time when the transition began, we have given in table 20 (page 101) a comparison of the rectal temperatures and the respiration rates of these two animals. The respiration rates were counted before the animals were disturbed by the taking of the temperatures, but it is believed that often the animals were disturbed by the mere entrance into the laboratory of the operator. The results show that when there is a high rectal temperature there is, in general, a high respiration rate and that both the rectal temperature and the respiration rate undergo pronounced changes during the transition stage as the animal is entering hibernation. However, with one and the same marmot at any given rectal temperature level there is no definite relationship between body temperature and respiration rate.

One feature of both the rectal temperatures and the respiration rates of these marmots during hibernation is the fact that there is not a continuous decline in these functions from the non-hibernating level to the level of deep hibernation. There is a considerable variability. The respiration rate and the temperature may both descend, then rebound to a certain extent, and then descend a little more later on, all of which can be interpreted as representing the extreme sensitivity of the marmot, when seemingly torpid, to external stimuli. This process of going into hibernation is of several days' duration. It is perhaps not surprising that there are slight ups and downs in the levels of the various life processes. The behaviour of the marmot entering hibernation is in striking contrast to its behaviour when waking up. In the latter case the animal's functions usually increase continually in activity from their lower levels to the awake levels, although there are some exceptions to this general procedure.

The variability of the respiration rate of the marmot in partial hibernation is important in showing whether this function can be used as an index of the condition of the animal. If the fall in rate is uniform, it may possibly be correlated with the heat production or some other function. If the transitions are fast enough to be noted, they may show some definite trend of the animal as it enters the hibernating stage. In a series of 22 consecutive respirations by marmot A, the time at which each respiration ended was noted with a stopwatch, and the rate per minute was calculated from the interval required for each respiration. The frequencies, expressed as the rates per minute, were as follows:

Resp. No.	Rate per minute	Resp. No.	Rate per minute
1	25	12	4
2	10	13	3
3	7	14	14
4	9	15	2
5	4	16	1
6	5	17	2
7	8	18	6
8	10	19	3
9	17	20	9
10	7	21	5
11	3	22	3

They range from 1 to 25 respirations per minute. The entire 22 respirations occurred in 5.18 minutes, averaging 4 per minute. Although the marmot was apparently in deep stupor, a striking wave-like effect in the rate is to be noted, *i.e.*, each temporary lowering in rate is followed by a rate of 9 or above. The observer did not touch the animal but simply entered the laboratory and began the observations as soon as possible. With the environmental temperature at 13.4° and the animal lethargic, there was a tremendous variation in the rate from respiration to respiration. Is it conceivable that there is a corresponding variation in the metabolic rate? If so, this would be very remarkable. The data cited above, therefore, can be taken as further evidence of the unreliability of the respiration rate as an exact index of the depth of hibernation or the intensity of the metabolism.

Of special interest in showing that the marmot was actually going deeper and deeper into hibernation are the respiration rates noted while a marmot was on the balance during the process of determining the insensible losses in weight. The respiration rates were as follows:

Time	Rate per min.
10.35 a.m.	8
11.45 "	7
12.40 p.m.	5
1.27 "	4
3.18 "	1

The final rate of 1 per minute would indicate deep hibernation, although the rectal temperature at this time was as high as 22.8° C.

Although it has been shown that the respiration rate is not an exact index of the rectal temperature and, as will be subsequently shown, is not closely correlated with the heat production, it does have some value. In view of the fact that it is the only function that can be observed without either handling the animal or previously attaching equipment such as heart rate electrodes or rectal thermo-junction, the presence of which may prevent hibernation, if this function has any value it should be recognized. The fact remains that the general trend of the respiration rate is to decrease as the marmot enters the hibernating stage, and as it goes into deeper hibernation the rate reaches at times a strikingly low level. This generalization holds true, as can be seen from table 31 (page 139) which shows the observed conditions and the respiration rates of 12 marmots over a representative period of 8 days. These respiration rates could be used to predict the condition of the animal with a reasonable degree of accuracy. Thus the respiration rate is a crude index of the condition of the marmot.

HEART RATE

The determination of the heart rate, although desirable, necessitates such distinctly disturbing contact with the animal that this measurement is ruled out in practically all cases where there is a transition from awake to hibernation, although it would be useful. When the electrodes could be attached to the marmot in deep sleep, the course of the heart rate during waking could

be studied as frequently as desired, but the application of the electrodes to the animal even in deep hibernation was frequently a stimulus to waking. Even when the marmot was not awakened, the attachment of the electrodes was always a disturbing factor for some time afterwards, making uncertain the records obtained. On one occasion it was possible to attach the electrodes when the marmot was awake and fortunately they remained intact, thus enabling one series of observations of the heart rate of a marmot when entering hibernation. As the respiration rate, rectal temperature, and heat production were also measured simultaneously on this occasion, the discussion of the heart rate will be postponed until the heat production is discussed. (See page 109.)

GASEOUS METABOLISM RESPIRATORY QUOTIENT

The fragmentary data secured in the periods of gaseous metabolism measurement on the marmot in the transitional stage from waking to sleeping (when there were profound changes in the metabolic activity) leave the picture as to the respiratory quotient very uncertain. Prior to entering hibernation the marmot had always been fasting, and hence the prevailing quotient was that of fat. Occasionally low quotients were found which challenged our attention. A fuller discussion of the respiratory quotient of the marmot in both the hibernating condition and the entering stage will be given later. (See pages 120 et seq.)

METABOLISM DURING THE TRANSITION INTO HIBERNATION

In the relatively few cases where the marmot could be studied in the transitory state the data secured are particularly valuable as showing the rates of such factors as heart rate, respiration rate, and metabolism. Not until the experiments in December 1936 could all these factors be simultaneously measured. The first fairly complete observations reflecting the transition stage were of marmot F on December 20, 22, and 23, 1932. These data, shown in table 21, were obtained in periods of approximately $1\frac{1}{2}$ hours each, which was a sufficiently long time to establish the metabolic level. The rectal temperature was taken before the experiment, unfortunately, for this procedure undoubtedly increased the metabolism somewhat. The result is that on each day there was an initial high value for the heat production, followed by decreasing values which reached about 200 calories or under on the last two days. The rectal temperature likewise decreased, beginning at 34.1° on the first day and ending at 21.3° on the last day. As each period was $1\frac{1}{2}$ hours long, these observations afford clear evidence of a descending metabolism as the marmot goes into hibernation, which latter fact is demonstrated by the rectal temperature. This change in metabolism may be quite rapid. This was notably the case in the experiment of December 23, when the level of heat production in the first $1\frac{1}{2}$ hours was 1680 calories, in the

next $1\frac{1}{2}$ hours 390 calories, and in the next two periods essentially constant. The average of the last two periods, 225 calories, at a rectal temperature of 21.3° is somewhat higher than the period on the day before when the rectal temperature was 23.4° , but one might argue that with an animal of this type the heat production at a rectal temperature of 21° would be not far from 200 calories per $10w^{\frac{3}{4}}$.

TABLE 21—*Metabolism of marmots entering hibernation*

Marmot	Date	Body weight	Temperature		Heat production per $10w^{\frac{3}{4}}$ per 24 hrs.
			Environmental	Rectal	
B	Jan. 6, 1932	1.76	kg.	°C.	cal.
					1530
					1090
					770
					800
	Feb. 1, 1932	1.52	10	°C.	570
					650
					540
					480
					540
F	Dec. 20, 1932	1.47	9	34.1	910
					1010
					640
					450
					400
	Dec. 22, 1932	1.45	9	27.1	230
					1160
					820
					700
					550
3	Dec. 23, 1932	1.36	9	28.3	1260
					960
					590
					160
					1680
	Dec. 23, 1936	2.13	9	21.9	390
					220
					230
					21.3
					1410
	Dec. 24, 1936		13 11	26.3 24.3	1170
					550

With marmot B in January and February, 1932, two experiments were made, with periods approximately three-quarters of an hour long. The rectal temperature was not taken, so there is no evidence of the rapidity of transition of this function as the marmot went into hibernation. The initially high metabolism on January 6 and in the first two periods on February 1 may be ascribed to the handling of the animal when placing it in the respiration chamber, although rectal temperature measurements were not attempted.

As the environmental temperature was essentially the same as with marmots F and 3, it can be assumed that B's rectal temperatures were not far from theirs. Only at the end of February 1 did B's metabolism decrease to the level of 200 calories reached by F. The experiment of February 1 with marmot B, made early in our series, was defective inasmuch as frequent rectal temperature measurements were not secured and the experiment was not continued as long as it should have been. Nevertheless it does show that marmot B began the day with a metabolism of approximately 1000 calories per $10w^{\frac{2}{3}}$ at an environmental temperature of 10° and that in successive periods, approximately 45 minutes long, the metabolism decreased until in the last period it was 230 calories. It is obvious that a marmot having as low a metabolic level as 400 and 230 calories at a temperature of 10° must be entering hibernation. The observer's records indicate that B was inert at the end of the experiment but was awake on the following day.

Throughout the series with both F and B the environmental temperature remained essentially 10° . The experiments with marmot F were made on the 8th, 10th, and 11th days of fasting. Animal B had been fasting 34 days prior to the experiment on January 6.

Perhaps the most important picture of the marmot going into hibernation was obtained in a study with No. 3 on December 23 and 24, 1936. With this animal continuous records were secured of the heart rate, the respiration rate, and the rectal temperature, but the water-vapor output was not determined. In these experiments the periods were, however, only about 10 to 15 minutes long. The process of entering hibernation step by step is demonstrated by the data on No. 3. The metabolism, measured at three different rectal temperature levels, remained constant at each temperature level, the heat values reported being the averages of three or more periods. On December 23, in the forenoon, when the rectal temperature was 26.3° and the chamber temperature 13°C ., the heat production per $10w^{\frac{2}{3}}$ was 1410 calories, the respiration rate was 20, and the heart rate, 133. In the afternoon, the rectal temperature had fallen to 24.3° , the chamber temperature to 11.3° , and the heat production to 1170 calories; the respiration rate was constant at 25 per minute, and the heart rate was 116. The next morning, December 24, the rectal temperature had fallen still further to 15.7° , as the refrigerator in which the marmot was kept had been lowered to 4.4°C . The heat production had diminished to 550 calories, the respiration rate to 7, and the heart rate to 51. Thus the picture is clear that during this period of transition the rectal temperature was falling, the metabolism was falling, the respiration rate in general was falling (although this is not so clearly shown), and the heart rate was falling. It is unfortunate that more observations of this nature could not have been secured. This evidence for No. 3, together with that given for F and B, proves unmistakably that all these physiological processes, if uncontaminated by disturbing factors, decrease in intensity during the transition from awake to hibernation.

WATER VAPORIZED FROM THE LUNGS AND SKIN

Because of the ever-present difficulty of having marmots during the transition stage under suitable conditions for measurement and because of the recognized pitfalls in measuring the water-vapor output of the marmot in a steady state, let alone a transitory condition, there is a great scarcity of data on the water-vapor output in the entering state.

Every precaution was taken to secure uncontaminated water-vapor measurements on the three days when F was studied in the transition stage. The measurements obtained show that the output of water vapor followed the course of the heat production, so that the percentage of heat lost through the path of water vapor did not undergo marked alteration throughout the entire series. The average loss of heat in this manner amounted to about 9 per cent. At the low heat-production level, therefore, accompanying the low rectal temperature the percentage of heat lost in water vapor by the marmot when entering hibernation is not pronouncedly different from that of the non-hibernating marmot at 16°, when its heat production and its rectal temperature are both higher.

The discussion thus far has dealt solely with the transition of the marmot by normal processes into normal hibernation, in which phase the hand of man played a rôle only in altering and maintaining the environmental temperature. The temperature of the room or garage in which the marmots were kept could be altered at will, and in the case of No. 3 the temperature of the refrigerator could likewise be adjusted. Other than this the animal simply followed out its own physiological needs. In subsequent sections of this report (pages 172 and 219) the picture of a transition period will be presented in studies using either nembutal or carbon-dioxide narcosis.

GENERAL CONCLUSIONS REGARDING THE TRANSITION INTO HIBERNATION

To determine the exact degree of hibernation of the marmot, one should have at hand several measures. The respiration rate in deep hibernation is obviously much lower than in the awake or in the transition state. The heart rate is low during hibernation and, aside from the difficulty of measuring it, especially when the marmot is awake, is a good index of the metabolic level. The record of the rectal temperature is likewise a valuable index but again not easy to measure. Perhaps the best index of all is the actual heat production or gaseous metabolism, again a factor not readily measured on a large number of individual marmots. In interpreting the results one must always be certain as to what degree of torpor and what levels of rectal temperature, heart rate, respiration rate, and especially metabolism the marmot has when entering hibernation. These measurements are particularly important for comparison with measures on the marmot awakening from hibernation.

Marmots passing from the awake condition into hibernation may do this by two different methods. When the marmot is first changing to this state

in the fall or when the tendency to hibernate comes into its yearly experience, there is rather a slow process of dips in the levels of its life processes. The first time for the season the animal never suddenly undergoes a change from a completely awake condition to complete torpor. In other words, at an environmental temperature of 10° the rectal temperature and the metabolism of the marmot do not change from 36° and 1000 or 1400 calories per 10w³, respectively, to 10° or below with a very low metabolism in 24 hours. Usually it takes several days for such decreases, at times a week or more. A striking illustration has already been noted in No. 3, the rectal temperature of which decreased to 26°, to 24°, and finally to 16°C. This may be assumed to be characteristic of the initial hibernating process, that is, for the marmot to go into deep stupor gradually. The second process is one that has been frequently observed, that is, after the marmots have been in deep hibernation at least once and either have waked normally or have been awakened by accident or by design, they can enter hibernation again, acquiring a low rectal temperature within 24 hours, indeed possibly less than that.

PHYSIOLOGY OF THE MARMOT IN HIBERNATION

The physiology of the non-hibernating marmot has already been considered and at least the general trends of the functions of the marmot as it enters hibernation, trends that hint as to the low level the marmot finally reaches when it is in deep hibernation. No one phenomenon is more striking with the marmot than the apparently complete absence of vital activity in deep hibernation. This animal may be one day awake and fiercely aggressive and the next day be utterly inert and approaching the level of a cold-blooded animal. As there are an infinite number of stages in entering hibernation, a sharp definition may not cover all. There is every stage from seeming drowsiness to partial lethargy and, finally, complete immobility. These are characterized by various physical phenomena. Consideration has already been given to the numerous steps or changes in the vital functions exhibited by the marmot when entering hibernation, that is, when passing from the warm-blooded state toward the cold-blooded state. Most of these functions are not going on at a constant level, for the marmot at this stage is continually changing the intensity of its vital activity. Even entering hibernation is not a continuous process, for the animal's life functions may slow down to a certain extent, then rebound, and then slow down again. Similarly in the waking stage, a slight stimulus might produce a slight quickening of vital activity followed by complete lethargy. But in the complete, profound torpor of hibernation the marmot is in a condition more nearly approximating that of true cold-blooded animals. A study of the marmot in this unique condition was one of the special features of our research.

In the earlier literature phenomena have been recorded which suggested that in hibernation the marmot has a greatly perturbed metabolism, characterized by an extremely low respiratory quotient at times reaching the level of 0.3 or 0.4.¹ Along with this has frequently been observed an actual gain in weight in the body during hibernation. From these two recorded phenomena it was maintained that during hibernation the oxygen-poor body fat, which the animal has in excessive amounts, undergoes a chemical transformation by being converted into oxygen-rich carbohydrate. If this process takes place to any considerable degree it must affect the respiratory quotient, that important measure of the relationship between the volume of carbon dioxide produced and the volume of oxygen consumed. The earlier observations implied that there is a great consumption of oxygen during hibernation, so great that there is an absorption and retention of oxygen in the body in sufficient amounts not only to offset the normal weight losses, which are in large part water, but to result in a gain in weight. This increase in body weight has been the subject of much discussion and will be considered more

¹ Conybeare, E. T., and M. S. Pembrey, Guy's Hospital Reports, 1926, 76, p. 342; Pembrey, M. S., Journ. Physiol., 1901, 27, p. 66.

in detail in another place. (See page 114) The old concept was that during hibernation there is an excessive absorption of oxygen by the body for the transformation of fat into carbohydrate, and that this increase in oxygen storage is sufficient to result in a positive increase in body weight of an animal taking neither food nor water. Thus it is seen that the magnitude of the respiratory quotient and the changes in body weight of the hibernating marmot are closely interwoven, and one of the most important studies in this research was the establishment of the true respiratory quotient during hibernation.

A second feature of our research was to study the extraordinary fall in rectal temperature experienced by these marmots during hibernation, with special reference to the rapidity of fall, the depth of fall, the lowest level to which the temperature can descend and the animal still survive, and ultimately the analysis of our data to note whether there are any correlations between the rectal temperature, the heart rate, the respiration rate, and more particularly the metabolic level.

A third salient point in our study was the establishment of the lowest metabolic level of the marmot at which it can exist and from which it can later be revived. It has already been seen in the study of the non-hibernating marmot that the basal metabolism at a rectal temperature of 37° and an environmental temperature of 28° is not far from 400 calories per $10w^{\frac{1}{2}}$ per 24 hours, a low metabolism for an animal with an average weight of not far from 2.5 kg. However, the establishment of this average basal level of the marmot tends to, but does not completely anchor the bridge between the warm-blooded and the cold-blooded animals at the warm-blooded end. The question then arises as to how closely the marmot's metabolism approximates that of the cold-blooded and how firmly it can be associated with that of the cold-blooded animal. To answer this question a complete survey of a number of animals in hibernation is necessary. Does the hibernating animal's metabolism reach the same low level as that of the cold-blooded animal? Are there wide differences in the levels? How low can the marmot's metabolism descend and the animal still be able to rebound? Finally, more important than all other questions, is the metabolism of the hibernating animal at its lowest level really in contact with, so to speak, the metabolism of the true cold-blooded animal?

In some of our experiments on hibernating marmots simultaneous measurements were made of a number of functions, heart rate, respiration rate, rectal temperature, and metabolism. How are these associated one with the other, when the animal is at its lowest level of heat production? These queries apply to that phase of the animal's life when it is in deep hibernation. There were a number of instances where the marmots were lethargic but did not have an extremely low heart rate, rectal temperature, or metabolism. These animals have been classified as in the transitory stage.

The physiological properties of body weight changes *per se* during hibernation, the insensible perspiration, the respiration rate and the various factors

affecting it, the heart rate, and the rectal temperature, all need special treatment. On the general principle that the metabolism of the marmot in hibernation approximates, if not reaches, the metabolic level of the cold-blooded animal, one can predict that these various functions will be much lessened in intensity.

BODY WEIGHT CHANGES OR INSENSIBLE PERSPIRATION

As the earlier observers have laid great stress upon the body weight changes during hibernation and have interpreted certain of the qualitative metabolic activities in the light of the apparent increases noted in body weight, a study of marmots with regard to their changes in body weight, especially when in deep hibernation, was imperatively necessary. The marmots when at the lowest point remained day after day immobile, without passing urine or feces. Hence the losses or changes in body weight could be determined with great accuracy upon a sensitive balance, such as the Sauter balance, which is capable of weighing 10 kg. or more with an accuracy of 10 mg. A large number of these observations were made. In view of what other writers had reported, we were looking particularly for gains in weight, the significance of which in relation to the respiratory quotient has already been touched upon. Strictly speaking, when an animal is receiving neither food nor water, living in an environment of a relatively low humidity, one would expect invariably a loss in weight because of the vaporization of water from the lungs and skin of the animal. Indeed, over a period of 4 or 5 hours one would expect to find a significant loss in body weight. It is important to establish precisely the changes in weight of the hibernating animal, not only to obtain an approximate measure of the water vaporized but particularly to note whether under any conditions there is a gain in weight. If so, can this gain be interpreted in any way other than that it is ascribable to the retention of oxygen, thereby favoring the belief that during hibernation there normally exists a very low respiratory quotient? Realization of these problems led us to lay special emphasis upon precise weight measurements.

In hibernation the marmots were, for the most part, inert and were weighed in a cold garage during the period from December 23 to February 4. The dry bulb temperature ranged only from 5.4° to 13.5°C., remaining for the greater part of the time close to 9°. The wet bulb temperature was not far from 7°, so that the relative humidity was uniform at about 65 per cent. The periods of study were usually 4 to 5 hours long, one in the morning, immediately followed by one in the afternoon. Not infrequently there was an overnight period, 15 to 16 hours long. No period was less than 2 hours.

The majority of the data on weight changes were obtained on marmots D and E, which were in hibernation over a considerable period of time. The rectal temperature, determined nearly every day after the last weighing, served as the index of the depth of hibernation. With marmot D on several occasions the rectal temperature was 25° or above, on one day reaching 36.0°, when the animal can not be considered to have been hibernating. In this

series of weighings we were particularly careful to note the precise changes in weight in the various periods. The marmot was examined carefully to see that it was dry at the start of the period, that no urine or feces were passed, and that no significant movements of its body occurred during the period

TABLE 22—*Changes in body weights of hibernating marmots D and E*

Marmot and date ¹	Length of period ¹	Loss (-) or gain (+) per 24 hrs.	Rectal temperature	Marmot and date ¹	Length of period ¹	Loss (-) or gain (+) per 24 hrs.	Rectal temperature
<i>1932-1933</i>							
<i>Marmot D</i>				<i>Marmot E</i>			
Dec. 24	2 $\frac{1}{2}$	-0.94	8.1	Dec. 23	7	-2.12	
" 25	22	.18		" 24	16 $\frac{1}{2}$	+.46	
" 25	12	+.87		" 24	3	+.15	
" 26	12 $\frac{1}{2}$	-.29		" 25	22	+.71	
" 27	21	-1.10	10.4	" 25	12 $\frac{1}{2}$	+1.39	
Jan. 11	6	-.52	8.9	" 26	12 $\frac{1}{2}$	-.39	
" 12	16	-.07	10.8	" 27	21	-.76	
" 12	8	-2.26	11.0	Jan. 5	3	-.69	11.9
" 13	16	-2.14		" 6	16	-.96	10.4
" 13	8	-.30	8.5	" 6	8	-1.34	13.6
" 14	16	-.59	7.3	" 13	6 $\frac{1}{2}$	-.43	8.4
" 18	6 $\frac{1}{2}$	-1.56	9.3	" 14	16	-.51	7.2
" 19	17	-7.02	36.0	" 16	6 $\frac{1}{2}$	+1.19	10.0
" 19	3	-9.00	34.9	" 17	16 $\frac{1}{2}$	-.04	7.9
" 20	16 $\frac{1}{2}$	+1.28	9.6	" 17	7	+.49	9.2
" 20	6 $\frac{1}{2}$	+1.33	10.2	" 18	16 $\frac{1}{2}$	-.43	9.0
" 21	16	-.96	7.9	" 18	7 $\frac{1}{2}$	-1.62	9.5
" 23	3 $\frac{1}{2}$	-2.06	10.0	" 19	17	-.64	7.9
" 23	3	-1.88	11.6	" 19	7	+.69	8.5
" 24	16 $\frac{1}{2}$	-6.25	26.6	" 20	16 $\frac{1}{2}$	-.14	8.6
" 24	1	-9.11	27.7	" 20	6 $\frac{1}{2}$	-4.88	13.9
" 24	2	-10.71	23.9	" 20	5 $\frac{1}{2}$	-21.99	
" 24	2	-2.09	23.9	" 21	11 $\frac{1}{2}$	-10.11	24.9
" 24	1 $\frac{1}{2}$	0.00	22.4	" 27	7	-1.43	8.6
" 25	15 $\frac{1}{2}$	+.69	9.4	" 28	16	-.38	7.1
" 25	5 $\frac{1}{2}$	+.13	8.7	" 30	2 $\frac{1}{2}$	-2.38	6.0
" 25	2	+.57	9.0	" 30	4	-.96	7.4
" 26	16 $\frac{1}{2}$	+.39	7.6	" 31	16	-.44	6.3
" 26	4	-.18	8.1	" 31	3	+1.07	7.3
" 26	3 $\frac{1}{2}$	-.32	8.4	" 31	4 $\frac{1}{2}$	+.16	8.8
" 27	16	-.37	7.6	Feb. 1	16	-.89	6.5
" 27	8	-.46	8.0	" 1	5	-1.70	7.1
" 28	16	-6.23	33.1	" 1	2 $\frac{1}{2}$	+.90	7.7
				" 2	16 $\frac{1}{2}$	+.07	8.2
				" 2	4 $\frac{1}{2}$	-.41	9.5
				" 2	3	-2.43	10.8
				" 3	16	-15.00	
				" 3	3	-.79	19.1
				" 3	5	-.23	15.3
				" 3	5	+.09	10.7
				" 4	13	+.20	6.4

¹The date given is the date when the period of measurement ended. In some instances the period started on the preceding date.

of measurement. The changes in weight are reported in terms of the loss or gain per 24 hours. In a number of instances (see table 22) an actual gain in weight was observed. This finding is of striking importance, as it agrees with earlier findings reported in the literature. In the case of marmot D

actual gains in weight were noted in eight periods, all of substantial length. Similarly with marmot E, which was measured over a longer period of time, gains in weight were observed in thirteen instances. With D these gains were observed on 4 days and with E on 10 days. Frequently the actual measured gain was not very great, but expressed per 24 hours it was occasionally significant. For example, there was a gain in the case of D of 1.33 gm. per 24 hours and in the case of E, 1.39 gm. The precautions taken in these weighings left no question as to the fact that the animals had gained in weight during this time. Although on one occasion both marmots gained weight on the same day, December 25, in general this was not true. On the contrary, on December 24 and on January 19 E gained and D lost weight and on January 20 the situation was reversed. On those days when gains were noted, there was no instance of a significantly high rectal temperature. In other words, the animals were in deep hibernation, judged from the standpoint of body temperature.

Because during these weighings the humidity, although reasonably constant, nevertheless did actually change, and because the marmots were heavily furred, the thought arose as to whether the differences in humidity might affect the amount of water absorbed or retained by the fur, under which conditions humidity would naturally play some rôle in the body-weight changes. According to Johnson,² the increases in body weight in hibernation were attributed by Valentin,³ as early as 1857, to absorption of moisture by the fur. This possibility was also recognized by Mareš⁴ in 1892 and Dubois⁵ guarded against it in his work. During the measurements made on D and E overnight from December 24 to December 25, at an environmental temperature of 6°, the humidity increased noticeably from 76 to 88 per cent, and both animals actually gained in weight. The humidity, however, did not usually increase markedly on those days when positive gains in weight were observed.

Two methods of studying the influence of prevailing humidity upon the weight of the animal were available. A piece of fur was molded about a wad of cotton, to form a dummy marmot, suspended on the balance arm in the garage, and left there for weeks. During this time it was exposed to the normal fluctuations in temperature and humidity of the garage itself, that is, to the conditions normally prevailing inside the garage, where measurements were made of the animals' insensible perspiration. There is no question but what a hibernating animal left on the balance for several days loses weight. On the other hand, it is conceivable that the fur covering of an animal, because of its innumerable hairs, might easily hold moisture by imbibition and that the amount would vary with the humidity. The piece of fur placed on the balance in the garage and left there for about 80 days was weighed

² Johnson, G. E., Quart. Review Biol., 1931, 6, p. 439.

³ Valentin, G., Moleschott's Untersuchungen, 1857, 1, p. 206; *ibid.*, 2, p. 1.

⁴ Mareš, F., Compt. Rend. Soc. de Biol., 1892, 9th ser., 4, p. 313.

⁵ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, pp. 103 et seq.

from day to day for several weeks. It was frequently noted that positive gains in weight took place, gains that in a 4-hour period would amount to a maximum of from 30 to 40 mg. In fact, expressed per 24 hours a gain of 0.18 gm. or more was observed on twelve different occasions from March 22 to April 14.

TABLE 23—*Changes in body weights of two dead marmots and a dummy marmot (4-hour periods)*

Date	Loss (−) or gain (+) per 24 hours		
	E gm.	G gm.	Dummy gm.
1933			
March 20		±0.00	
" 21		-.24	
" 22	+1.68	+2.40	
" 23	+1.92	+.12	
" 24	+.72	.00	
" 25	+.60	-.36	
" 26	+.30	.00	
" 27	+2.40	.24	
" 28	+.78	.00	
" 29	+.42	.12	
" 30	+1.92	-.12	
" 31	+.78	.30	
April 1	+.60	-.30	
" 2	-4.02	-1.80	-1.62
" 3	-.30	-1.92	-1.44
" 4	-3.30	-1.30	-.06
" 5	-.14	-1.92	-.48
" 6	+.18	-.42	
" 7	±.00	-.18	
" 8	±.00	-.42	
" 9	-.30	.00	
" 10	-3.0	-.48	
" 11	-1.68	-.48	
" 12	-.12	-.24	
" 13	+.42	-.30	
" 14	-1.14	-.18	
" 15	+.42	-.78	
" 16	-.30	-.48	
" 17	-1.14	-.24	
" 18	+.12	-.30	
" 19	-.12	-.18	
" 20	+.18	-.30	
" 21	-.12	-.48	
" 22	+.12	-.24	
" 23	-.12	-.30	
" 24	+.06	-.48	
" 25	-.12	-.24	
" 26	+.06	-.30	
" 27	-.06	-.48	
" 28	+.06	-.24	
" 29	-.06	-.30	
" 30	+.06	-.48	
" 31	-.06	-.24	
" 32	+.06	-.30	
" 33	-.06	-.48	
" 34	+.06	-.24	
" 35	-.06	-.30	
" 36	+.06	-.48	
" 37	-.06	-.24	
" 38	+.06	-.30	
" 39	-.06	-.48	
" 40	+.06	-.24	
" 41	-.06	-.30	
" 42	+.06	-.48	
" 43	-.06	-.24	
" 44	+.06	-.30	
" 45	-.06	-.48	
" 46	+.06	-.24	
" 47	-.06	-.30	
" 48	+.06	-.48	
" 49	-.06	-.24	
" 50	+.06	-.30	
" 51	-.06	-.48	
" 52	+.06	-.24	
" 53	-.06	-.30	
" 54	+.06	-.48	
" 55	-.06	-.24	
" 56	+.06	-.30	
" 57	-.06	-.48	
" 58	+.06	-.24	
" 59	-.06	-.30	
" 60	+.06	-.48	
" 61	-.06	-.24	
" 62	+.06	-.30	
" 63	-.06	-.48	
" 64	+.06	-.24	
" 65	-.06	-.30	
" 66	+.06	-.48	
" 67	-.06	-.24	
" 68	+.06	-.30	
" 69	-.06	-.48	
" 70	+.06	-.24	
" 71	-.06	-.30	
" 72	+.06	-.48	
" 73	-.06	-.24	
" 74	+.06	-.30	
" 75	-.06	-.48	
" 76	+.06	-.24	
" 77	-.06	-.30	
" 78	+.06	-.48	
" 79	-.06	-.24	
" 80	+.06	-.30	
" 81	-.06	-.48	
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" 84	+.06	-.48	
" 85	-.06	-.24	
" 86	+.06	-.30	
" 87	-.06	-.48	
" 88	+.06	-.24	
" 89	-.06	-.30	
" 90	+.06	-.48	
" 91	-.06	-.24	
" 92	+.06	-.30	
" 93	-.06	-.48	
" 94	+.06	-.24	
" 95	-.06	-.30	
" 96	+.06	-.48	
" 97	-.06	-.24	
" 98	+.06	-.30	
" 99	-.06	-.48	
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" 347	-.0		

averaging not far from 9° and 7° C., respectively. Weighings of two of the bodies continued for approximately a month and of the third body for eight days. At the same time the dummy or cotton-filled fur was weighed from day to day under precisely the same conditions. Thus records were secured of the changes in weight from day to day, to note whether there was a loss in weight as a result of the drying of the dead marmot's body or whether there were measurable gains. Furthermore, it was possible to compare the weights of the two dead animals measured at the same time. Finally, comparisons were made with the changes in weight of the dummy animal at the same time. These data for the two marmots weighed over a period of a month and for the dummy marmot are presented in abstract form in table 23, the actually observed changes in weight during 4-hour periods being reported in terms of 24 hours for purposes of comparison. Although we were dealing with two dead animals, apparent gains in weight were frequently noted as well as losses in weight. In the course of 23 days, however, the body of E lost a total of 8.38 gm. and that of G, 8.11 gm. It is thus obvious that these gains in weight are purely of a transitory nature and are even more than compensated for by the losses. As a matter of fact, G weighed at death but one-third the weight of E, so the chief value of table 23 is the evidence it affords of the actual gains in weight without any attempt to record quantitatively the day-to-day status of the dead animal's weight. In general, as would be expected, the trend of the weights was much the same with both animals. When one animal gained, the other either had no loss or had a slight gain in weight, and *vice versa*. This was by no means invariably the case but was the general picture.

A still further analysis can be made by comparison of the fluctuations in weight of E and the dummy marmot. When gains are noted with E, there are almost invariably gains with the dummy. In fact, the agreement from period to period is striking. Especially is this noted on days where there are pronounced losses with E and similarly pronounced losses with the fur. On March 29 the 24-hour loss is 4.02 gm. with E and 1.62 gm. with the dummy. On April 5 there were losses of 1.68 gm. with E, 1.14 gm. with G, and 0.30 gm. with the dummy. It is a fact that a hibernating marmot may gain in weight, but the same may be said with regard to a dead marmot, and the same phenomenon is observed with a piece of fur maintained under the same atmospheric conditions. As a matter of fact, the gains in weight per kilogram of the dead animals are frequently greater than the maximum 24-hour gains observed with hibernating marmots. It is thus clear that gains in weight of the hibernating marmot can not be ascribable to the absorption of oxygen, for similar gains are found with a dead animal and with fur.

A short experiment lasting but eight days was made with another dead marmot and showed precisely the same picture, *i.e.*, gains and losses in weight, which were likewise reasonably closely associated with the changes in weight of the dummy.

Finally in another series a year later, two hibernating marmots, I and K,

were weighed precisely from hour to hour on March 7 and March 8. (See table 24.) In one period with marmot I there was a gain (calculated per 24 hours) of 0.64 gm., and in four periods with K, each about an hour long, gains were also noted. The respiration rates were very low, indicating that the marmots were in deep hibernation. Thus the evidence is clear that the gain in weight of the hibernating marmot is not an uncommon finding. The evidence is equally clear that this can not be due to oxygen storage but that, according to our experience with dead animals and with fur, it is primarily, if not entirely, ascribable to the hygroscopic material being weighed.

This reminds one of the experiences in various clinics in Europe, where measurements have been made of the insensible perspiration of humans, as recommended by the Nutrition Laboratory. A visitor from this Laboratory found these experiments being made with patients lying upon hair mattresses,

TABLE 24—*Changes in body weights of hibernating marmots*

Marmot	Date	Body weight	Length of period	Loss (-) or gain (+) per 24 hrs.
I	1934 March 7	1.30	hr.	gm.
			1	+0.64
			1	-2.43
			1	-1.12
			1	-.12
K	March 8	1.13	hr.	gm.
			1	-1.02
			1	+.66
			1	+1.07
			1	-.14
			½	+5.52
			2	-.12
			1½	+.90

under which conditions not only was the insensible perspiration being measured but, as the writer⁶ expressed it, likewise the changes in weight of the equivalent of 10,000 hair hygrometers.

Because of the lability in the body weight of the marmot, dependent as it is not only upon the degree of hibernation (when the rectal temperature is above 25° the loss in weight is much greater than when the temperature is about 8° or under) but also upon the environmental conditions, the true insensible perspiration of the marmot in deep hibernation can only be approximately estimated. Not only may an increased humidity cause the animal to increase in weight but a decreasing humidity may contribute to a subsequent loss, which will result in too great an apparent loss because of the loss of imbibed moisture.

As there was considerable difference in the body weights of the two marmots, E weighing 2.0 kg. and D, 1.0 kg., comparisons of the changes in weight should be made on the basis of losses per kilogram of body weight, although to save space these calculations have not been tabulated. The gains in

⁶ Benedict, F. G., Zeitschr. f. d. ges. expt. Med., 1933, 91, p. 340.

weight are ruled out of the discussion, for it has been shown that these gains have no true physiological significance. With D the minimum true loss per kilogram per 24 hours was in the neighborhood of 0.3 gm., whereas with E, the heavier animal, there were several instances where the loss was under 0.2 gm. Marmots I and K, each weighing approximately 1.0 kg., also had losses below 0.2 gm. Thus for the marmot in deep hibernation it can be concluded that the insensible loss is approximately 0.2 gm. per kilogram per 24 hours. Although an average for the insensible loss of the marmot in deep hibernation is given, this value can not be of great significance as it is so profoundly affected by the changes in humidity.

These small weight losses do not occur unless the marmot is in deep hibernation. For example, on those days when the rectal temperature was 24° C. or over, the insensible losses were rather considerable, in the order of 6.0 gm. or more, per 24 hours. On one day, with E, January 20, the loss per 24 hours was 22 gm., but the rectal temperature was not taken at the time of this measurement. In two observations on D (definitely awake) on January 19, when its rectal temperature was essentially normal (36° C.), the loss per 24 hours was 7.02 and 9.00 gm., respectively. The average of these two values, 8 gm. per kilogram of body weight (D weighed 1 kg.), at a rectal temperature of 35° or 36° C., is in agreement with the general average of 8.4 gm. per kilogram per 24 hours noted with non-hibernating marmots at an environmental temperature of 16° C.

In those periods when the rectal temperature was lowest (6° to 8° C.) and the losses were minimum, the loss per kilogram per 24 hours of a marmot in deep hibernation may be stated to be 0.2 gm. From the standpoint of studying the physiology of hibernation a comparison is necessary of this level of insensible perspiration, which is practically vaporized water, with that under normal, awake, basal conditions. Inasmuch as at an environmental temperature of 16° the loss was 8.4 gm. and at 31°, 16.1 gm., the deep hibernating level is less than 3 per cent or one-fortieth that at 16°, and one-eightieth that at 31° C. In many of the earlier reports the significance of gains in weight has been grossly exaggerated, and around such gains the story is built that the animal is storing oxygen which is subsequently released when the animal awakens. Because the relatively small changes in hygroscopic conditions may be such as to offset completely the normal loss in body weight during hibernation of 0.2 gm. per kilogram per 24 hours and to show actually an increase in weight, and because increases in weight of this order of magnitude are reproducible with the dead marmot, it is obvious that, based upon this type of evidence, no further discussion of oxygen retention and conversion of fat to carbohydrate by the hibernating animal is justifiable.

RESPIRATORY QUOTIENT (COMBUSTION QUOTIENT)

Perhaps no one point is more important physiologically than a consideration of the supposed qualitative changes which the metabolism is said to undergo as a result of hibernation, the evidence for such qualitative changes

being the large number of aberrant respiratory quotients quoted in the literature. Under normal conditions a fasting animal will, for an almost indefinite length of time, have a respiratory quotient of fat, *i.e.*, about 0.71.⁷ Any animal deprived of food quickly reaches a metabolic state where the respiratory quotient is that of fat. With continued fasting the quotient usually remains at this level, save in the rare cases where the deprivation of food is prolonged to such an extent that the animal depletes its fat reserve and drafts upon body protein are necessary. Under these conditions the respiratory quotient rises from that of fat and approaches 0.81, that of protein. This usually occurs only just prior to death, and in but a few cases has it been actually observed. However, the reporting of respiratory quotients below the fat level is, for hibernating animals, a common occurrence, an extreme case being the inconceivably low value of 0.4 for the marmot reported by Pembrey.^{8a}

For a study of the respiratory quotient alone it is not necessary to know the total volume of air ventilating the chamber, although it is absolutely necessary in case a measurement of the total heat production is desired. The true respiratory quotient, or more properly the combustion quotient, in the sense of Carpenter,^{8b} is the relationship between the carbon dioxide *produced* and the oxygen consumed. It may not be accurately measured by the carbon dioxide *given off* and the oxygen absorbed. The ordinary errors in sampling and analysis are such that with good technique they play no significant rôle when the respiratory quotient and the metabolism of animals under normal conditions are being measured. On the other hand, with the hibernating animal, where the gaseous metabolism sinks to an unbelievably low level, one is confronted instantly by a large number of possibilities for error, each one of sufficient magnitude to cloud the picture. This was too slowly recognized by us, although we were early aware of the great errors inherent in the measuring of the respiratory quotient of the hibernating animal by the Hal-dane method. As this method necessitates the weighing of absorbers and particularly the weighing of the chamber containing the animal to determine the weight changes, possibly jeopardized by the condensation of moisture on the exterior walls of a good-sized chamber which is only partially compensated for by the use of a dummy chamber, this method is unfitted for the measurement of the microscopic amounts of carbon dioxide produced by the 2-kg. hibernating marmot. Hence in this study respiratory quotients determined by the closed-circuit method, formerly used in the Nutrition Laboratory for the measurement of the oxygen consumption and occasionally the respiratory quotient, were discarded and a technique (already described, page 19) using the extremely precise Carpenter gas-analysis apparatus was

⁷ Throughout this manuscript this quotient or 0.70 is used as an indication of the fat quotient. It is obvious that rarely, if ever, is there an exclusive fat combustion in hibernation or during fasting. There is invariably a certain proportion of protein oxidized, which may represent not far from 10 to 15 per cent of the total energy production.

^{8a} Conybeare, E. T., and M. S. Pembrey, Guy's Hospital Reports, 1926, **76**, p. 342.

^{8b} Hendry, M. F., Carpenter, T. M., and L. E. Emmes, Boston Med. and Surg. Journ., 1919, **181**, p. 341.

relied upon. Provided that the sample of air to be analyzed on the Carpenter apparatus has between 0.5 and 1 per cent of carbon dioxide, there can be little criticism of the accuracy of the determinations of the carbon-dioxide increment and the oxygen deficit in the air sample. However, the accurate determinations of these do not by any means insure an accurate determination of the true respiratory quotient. That the increment in carbon dioxide and the oxygen deficit correspond precisely to the *production* of carbon dioxide and the consumption or absorption of oxygen in the same period is by no means true.

In the years in which this marmot research was in progress careful study was made of the errors in the determination of the respiratory quotient incurred by rapid changes in the carbon-dioxide content of the air in the respiration chamber and of the requirement of a rate of ventilation such as to obtain a suitable percentage of carbon dioxide in the gas to be analyzed. As carbon dioxide is soluble in water in measurable quantities, any system using a water-sealed chamber, a water-sealed sampling spirometer, or one in which water is present due to condensation of moisture or urine in the chamber, was considered unreliable for the determination of the respiratory quotient. During the development of the techniques innumerable respiratory quotients of hibernating animals by the various techniques were secured. When this material was assembled, the extraordinarily low values reported by others in the order of 0.5, 0.4, or 0.35 at no time appeared. All of our experiments in which the respiratory quotient was determined were surveyed, at the same time the techniques being examined closely, and all values were rejected in which the technique was apparently defective. These techniques, however, although unreliable for respiratory quotient determinations, did not vitiate the oxygen measurements used to calculate the total metabolism. With this treatment of the data a large proportion of the seemingly aberrant respiratory quotients automatically disappeared and along with them many respiratory quotients that frankly seemed quite normal. The resulting general picture showed a respiratory quotient for the hibernating animal substantially in the neighborhood of the fat quotient. (See table 26.)

At the conclusion of this survey there still remained a few experiments which gave seemingly positive evidence of the possibility of respiratory quotients as low as 0.61. The earliest low quotients, determined by the so-called "better technique", were noted with marmot I on March 14, 1934. (See table 25.) This animal, which had been hibernating since February 17, 1934, was placed in the respiration chamber at an environmental temperature of 9.0°C. Because the animal was slightly stimulated by being placed in the chamber, there was at the start of the experiment a rise in the metabolism, during which time three respiratory quotients of 0.75, 0.76, and 0.70 were noted. Following these, analyses of chamber air samples drawn intermittently over a 4-hour length of time gave respiratory quotients of practically 0.64. No technical error could be found in these analyses. The percentage of carbon dioxide was amply high for analysis on the Carpenter apparatus.

The technique was approved, a wax-sealed chamber and oil-sealed spirometers for sample collection being used. To be sure, the carbon-dioxide production per minute (0.5 c.c.) was extremely small, and the rate of ventilation for the chamber was only 100 c.c. per minute. The volume of air about the animal in the chamber was approximately 4 liters. Hence, theoretically, 40 minutes (really longer) were required to remove all the chamber air once. This in no way affected the results obtained, as a series of periods over a number of hours showed a uniform respiratory quotient. It is thus apparently

TABLE 25—*Apparent respiratory quotients of marmots in hibernation*

Marmot, body weight, days fasting	Date and time	Environmental temperature °C.	Measured respiratory quotient ¹	Heat production per $10w^{\frac{1}{3}}$ per 24 hours cal.
Marmot I 1.23 kg. 58 days	March 14, 1934			
	2.15 p.m.	9.0	.75	290
	3.14 "	9.0	.76	70
	4.00 "	9.0	.70	60
	4.46 "	9.1	.64	50
	5.47 "	9.0	.65	40
	7.33 "	9.2	.64	42
	8.08 "	9.1	.64	43
	11.08 "	20.8	.66	46
	11.27 "	22.0	.59	44
	12.52 a.m.	25.9	.69	170
	1.21 "	28.5	.70	270
	2.00 "	30.1	.83	150
	2.46 "	31.5	.74	2190
Marmot L 1.57 kg. 57 days	Jan. 10, 1935			
	11.36 a.m.	13.0	.70	80
	12.05 p.m.	12.5	.70	44
	1.20 "	12.3	.59	16
	1.57 "	12.4	.59	18
	2.33 "	12.4	.61	33
	3.58 "	19.2	.67	90
	4.32 "	25.5	.71	40
	5.22 "	27.5	.70	34
	6.10 "	28.7	.71	2760
Marmot 26 1.44 kg. 67 days	Jan. 27, 1937			
	2.58 p.m.	4.0	.66	33
	3.38 "	3.5	.66	34
	4.18 "	3.2	.67	32

¹ Periods 20 to 40 minutes' duration.

impossible to reject this experiment on the basis of experimental technique. Seemingly we have a good picture of a marmot maintaining a respiratory quotient of 0.64 for four hours.

If it is assumed for the moment that this represents a true respiratory quotient, that is, the true relationship between the carbon dioxide produced and the oxygen absorbed by the animal at this time, it is a strange situation that the respiratory quotient of the marmot in the first two periods was above 0.70 and then suddenly dropped to 0.64 and stayed there. It is highly unlikely that there was not some disturbing factor that made the first two quo-

tients seem to be higher than they really were. On this basis alone we would be justified in questioning whether they represent a true physiological fact. If the quotients below 0.70 are attributed to the absorption or retention of oxygen to convert an oxygen-poor substance, fat, into an oxygen-rich substance, carbohydrate (the popular interpretation of such a situation), then apparently for 4 hours marmot I absorbed enough excess oxygen to reduce the quotient from that of fat, 0.70, to 0.64. The actual amount of carbon dioxide eliminated was not far from 0.5 c.c. per minute, with a respiratory quotient of 0.64. Consequently the oxygen consumption per minute was 0.8 c.c. If the carbon-dioxide elimination is ascribed to a combustion of fat, the oxygen absorption for this process would be 0.7 c.c., which means that an additional oxygen consumption of but 0.1 c.c. per minute will lower the respiratory quotient from 0.71 to 0.64. Hence, in the 4-hour period during which the respiratory quotient remained at essentially 0.64, a retention of oxygen during this time (240 minutes) at the rate of 0.1 c.c. per minute would result in a storage of 24 c.c. Glycogen contains approximately 50 per cent of oxygen. The 24 c.c. of oxygen stored would represent approximately 34 mg. of oxygen. If it is assumed for simplicity's sake that the formation of glycogen starts not from fat with its approximately 12 per cent oxygen but from an oxygen-free substance, then as glycogen is one-half oxygen, 68 mg. of glycogen would be formed in the four hours. The heat of combustion of glycogen is about 4 calories per gram. Therefore, 68 mg. of glycogen would represent approximately one-fourth of a calorie. The earlier writers believed that the glycogen formed during the period of hibernation was drawn upon to aid in the rapid rewarming of the body during the waking period, when the oxygen consumption increased from 0.7 c.c. (calculated on the basis of a respiratory quotient of 0.70) to as high as 37 c.c. per minute. This high oxygen consumption continued for a 20-minute period, during which time there was consumed 740 c.c. of oxygen. Hence the heat produced in this time was about 3.7 calories, the energy value of a liter of oxygen being fairly constant at not far from 5 calories, irrespective of the substance burned. Thus the 68 mg. of glycogen theoretically formed in this fat transformation with its potential 0.25 calorie would furnish but 7 per cent of the energy needed during the intense metabolism of the 20-minute waking-up period. It is, therefore, not surprising that the participation of this small amount of glycogen is not apparent during the increased combustion of the waking-up and subsequent periods. As the matter now stands, it would appear that, by the establishment of a respiratory quotient of 0.64 for a period of 4 hours by a technique that is instrumentally impeccable, the possibility for such low quotients apparently is a reality. The one striking criticism of this is the fact that in two of the three immediately preceding periods the quotients were above 0.70.

The next similar case in which a low quotient was established by a seemingly perfect technique was that of marmot L on January 10, 1935 (see table 25). For three periods representing $1\frac{1}{2}$ hours the animal had quotients of 0.59,

0.59, and 0.61. In subsequent periods the quotient was exactly at the fat level, which was also the case during the waking-up condition. Any glycogen formed in the $1\frac{1}{2}$ hours of the seemingly glycogen-forming period should have been burned during this waking period, and hence we would have expected quotients higher than 0.70. In fact, as shown in table 25, marmot L had a metabolism during two hibernating periods but little above its deepest hibernating state, with a respiratory quotient of fat, and yet why the quotient increased suddenly from a level of practically 0.60 to that of 0.71 is puzzling. This increase may perhaps be associated with the rise in the environmental temperature from 12.4° to 28.7°C . The rectal temperature was not taken until the animal came out of the chamber five hours later, at which time it was 35.7° C .

Thus far, therefore, there are two experiments, one with marmot I and one with L, which offer a plausible justification for assuming that there has been an establishment of the existence of a respiratory quotient of 0.64 in one case and 0.60 in the other. These are reported not as accepted physiological facts but because of their connection with the work of earlier writers who were so strongly dominated by such findings as to let them color their whole interpretation of the processes in hibernation and, in the last analysis, it is this—the interpretation of hibernation—in which we are most interested.

Thus far in this discussion emphasis has been laid upon respiratory quotients lower than 0.70. With one marmot an aberrant respiratory quotient much above 0.70 was found. When No. 3 on December 28, 1936, had been fasting for 37 days, there were six periods in which there was a low caloric output of 75 calories per $10w^3$ per 24 hours and in which the respiratory quotients were at first 0.74, 0.72, and 0.74, and then rose rapidly to 0.78, 0.81, and 0.80. As the animal died five hours later, this sudden rise has been temporarily at least interpreted as a premortal rise in the metabolic drafts upon body protein. This is cited here simply as an instance of such rises following deep hibernation.

Realizing the importance of the respiratory quotient study and attempting to refine still further the technique, we planned an experiment on February 20, 1935, with L in which the animal was kept in the respiration chamber for 25 periods of approximately 30 minutes each and measurements made only of the respiratory quotient. The chamber temperature was held at about 10° , but continuous measures of the rectal temperature were not obtained. As a result of 25 measurements throughout these 12 or more hours the quotient underwent a variation from 0.68 to 0.77, averaging throughout the entire time close to 0.71. The animal had a respiration rate of 2 per minute at the end of the experiment and a rectal temperature of 11.1°C . Here a hibernating animal in a long series of consecutive periods had at no time a respiratory quotient significantly below 0.70, the average value being definitely above 0.70. The experiment was somewhat complicated by the fact that at the end, when the animal was taken out of the chamber, it was found that a considerable amount of water had leaked into the chamber, but it is believed

this leaked in near the end of the experiment. The last five quotients in this experiment were 0.73, 0.72, 0.71, 0.70, and 0.69. This experiment clearly indicates that, at least under these conditions, the gaseous metabolism was not such as to give a quotient other than that resulting from a predominantly fat combustion.

Numerous observations were obtained with No. 26 during hibernation, when its heat production was very low, 30 to 35 calories per $10w^{\frac{1}{2}}$ per 24 hours. In all these observations the quotient was invariably that of fat, with the single exception of the first day of the study, January 27, when for 3 consecutive 40-minute periods the respiratory quotient was 0.66, 0.66, and 0.67. (See table 25 and table 26.) In order to secure an air sample of suitable carbon-dioxide percentage for analysis, the ventilation rate had to be extra-

TABLE 26—*Apparent respiratory quotients of marmots, hibernating or under influence of nembutal*

Condition and marmot	Date	R.Q. ¹	Condition and marmot	Date	Time	R.Q.
Hibernating I	March 14, 1934	0.64	Under nembutal 24	Jan. 11, 1937	2.00 p.m.	0.64
L	Jan. 10, 1935	.59		" 12, "	10.20 a.m.	.65
	" 15, "	.74		" 13, "	3.42 p.m.	.67
	Feb. 14, "	.69		" 14, "	10.45 a.m.	.65
	Feb. 20, "	.71			10.26 a.m.	.66
					3.40 p.m.	.67
M	July 30, 1935	.61			10.30 p.m.	.67
	" 31, "	.61		" 15, "	10.35 a.m.	.69
	Aug. 1, "	.63		" 17, "	10.59 a.m.	.71
	" 2, "	.66		" 18, "	8.40 a.m.	.70
	" 3, "	.69		" 19, "	8.16 a.m.	.69
	" 5, "	.66				
3	Dec. 28, 1936	.74				
26	Jan. 27, 1937	.66				
	" 28, "	.71				
	" 29, "	.70				
	" 30, "	.70				
	Feb. 4, "	.70				
	" 10, "	.69				

¹ Average of two or more consecutive periods, each of from 15 to 60 minutes' duration.

ordinarily low, 2 liters in 40 minutes, or about 50 c.c. per minute. As the respiration rate was 0.7 per minute, the animal was undoubtedly in deep hibernation. No measurements on January 27 immediately before or after these periods were made. It is, however, worthy of note that on that same day samples of air drawn directly from the chamber gave respiratory quotients slightly above this average (aliquot sample) of 0.66, and a measurement at 11.46 p.m., or 7 hours after the third period of the respiration experiment, showed a quotient of 0.70 in place of the 0.67 in the third period. No. 26 was left in the chamber overnight with a ventilation slow enough to maintain the carbon-dioxide percentage at approximately 1.0. The respiratory quotients, as shown by analyses of samples drawn directly from the chamber in the morning, were 0.70 and slightly above. Thus far, three different ex-

periments (tables 25 and 26), one with marmot I, one with L, and one with No. 26 have shown low quotients. To offset this are a number of other measurements with L and No. 26 showing normal fat quotients.

The concept of a low respiratory quotient during hibernation receives greatest support in a series of experiments made with animal M on July 30, July 31, August 1, and August 2, 1935. (See table 27.) During this entire period animal M was hibernating and had respiratory quotients for the most part between 0.60 and 0.65. These experiments met all the requirements known at that time so far as the instrumental side or manipulation is concerned. (See page 19.) Marmot M was wholly unusual, however, in that

TABLE 27—*Apparent respiratory quotients of marmot M during hibernation*

Date and time	Respiratory quotient ¹	Date and time	Respiratory quotient ¹
1935			
July 30		Aug. 2	
1.16 p.m.	.60	11.11 a.m.	.62
1.34 "	.61	11.36 "	.64
1.53 "	.61	12.01 p.m.	.63
2.10 "	.62	12.26 "	.63
3.22 "	.61	12.51 "	.65
3.46 "	.61	1.16 "	.66
		1.56 "	.68
July 31			
10.04 a.m.	.61	2.25 "	.68
10.24 "	.61	3.10 "	.72
10.44 "	.60	3.41 "	.70
11.04 "	.61	Aug. 3	
		8.58 a.m.	.71
Aug. 1			
1.46 p.m.	.63	9.47 "	.69
2.11 "	.63	10.33 "	.73
2.36 "	.64	12.37 p.m.	.64
3.01 "	.65	1.25 "	.63
3.27 "	.64	Aug. 5	
		10.28 a.m.	.68
		10.48 "	.64
		11.13 "	.66
		11.43 "	.67
		1.43 p.m.	.68
Aug. 8			
		10.25 a.m.	.73

¹ Periods of approximately 20 minutes' duration.

it was the only one of our animals that hibernated in the summer. Hibernation of M in the summer was effected as follows. The animal was removed from food on May 15 and placed in a refrigerator, with water available. The environmental temperature was maintained at about 8°C. A wire cage approximately 140 mm. by 350 mm. by 140 mm. restricted the gross movements of the animal. A heavy black cloth completely covered the cage to produce darkness. On June 19 the marmot was first noticed as being drowsy or in the first stages of hibernation. This condition was observed frequently during the next ten days, and on July 1 the animal appeared to be hibernating. On July 7 the animal was awake but was hibernating on July 9.

Attempts to place M in a respiration chamber to measure the metabolism on this day resulted in the animal's awakening from the stimulus of handling. On July 24 it was placed in a respiration chamber ventilated mechanically at about 4 liters per minute, and this in turn was placed in a large metal box which was surrounded by a water bath. By replacing the water with salt and ice, an environmental temperature of about 10° C. was maintained. On July 30 the carbon-dioxide production was found to be very low, indicating that the animal was in hibernation. After the measurements on the following ten days, the marmot was placed in a wire cage in the refrigerator and maintained for a period of six weeks in hibernation. It is possible that it woke up occasionally, but generally it was hibernating.

That this hibernation in summer of itself should have any effect on the character of the gaseous metabolism is hardly conceivable but at least worthy of study. Although some of the other marmots studied, notably I and L, had low quotients just prior to waking up, marmot M and also No. 26 in its first experiment showed low quotients which were not followed by a waking phase. To be sure, extraordinarily low ventilation rates were needed to hold the carbon dioxide at the proper percentage for analysis, *i.e.*, about 0.70 per cent. Moisture condensation inside the chamber was inevitably present, but with M and No. 26 the complicating factor observed with I and L, of high quotients immediately preceding the appearance of the low quotients, was not present.

We have felt it important in the interest of scientific honesty to present these data, for they represent a series of experiments which, at the time they were made, had met every criticism in the way of instrumental technique and management that we could imagine. One point was overlooked, and that was the inordinately long time it takes for an animal to attain an equilibrium of carbon dioxide in the tissues when there is a change of carbon dioxide in the environmental air or a change in body temperature. As will be seen from further experiments with marmots under the influence of nembutal, in which the study of the respiratory quotient was the main feature, it is this period of readjustment of the equilibrium of the carbon-dioxide content of the tissues to that of the environmental air or to the tissues at a different cell temperature that wreaks havoc with the determinations of the respiratory quotient. Let us emphasize again, therefore, that the quotients recorded here are as found by us by an acceptable technique. They are not true physiological facts, but quotients considerably below 0.70 are reported so frequently by other writers that we believe they are worth noting here. These quotients are striking exceptions and most of our observations with marmots in deep hibernation give a consistent picture of a respiratory quotient characteristic of the combustion of fat.

One factor that limited the study of the respiratory quotient was that one can not get marmots in hibernation at will, and a marmot in deep hibernation frequently was awakened by the handling preparatory to the experiment.

Late in the experimental series, particularly to contribute to knowledge of the true respiratory quotient, the use of a narcotic was resorted to in order to produce artificially a condition simulating hibernation. In December 1936 experiments were initiated using nembutal. (See previous discussion on page 20.) The purpose was to be able to produce hibernation and, if possible, to maintain the other physiological functions normal, thus enabling extended careful studies of the marmot in deep hibernation. Uniform narcosis of our marmots was not obtained. It was found that the condition of the marmot previous to the injection played some rôle in the ultimate effect of the drug, and that previous food intake and previous environmental temperature may be responsible for some of the variability in results. Marmots drugged in the winter were fairly readily anesthetized by a dose but slightly larger than the recommended dose of 43 mg. per kilogram of animal, although in some cases an additional half dose was required. There is a hint that season plays a rôle for in the spring, although the animals were sometimes drugged two and three times, it was difficult to reach as deep a state of anesthetization as in the winter. Frequently heavily drugged animals stopped breathing at rectal temperatures just under 20° C. but could usually be revived by administering oxygen and artificial respiration and by quickly warming, provided these steps were taken within a few minutes after respiration ceased.

The marmots that were drugged were almost invariably subjected to a cold temperature, in one or two cases to a room temperature of 24° C., but usually temperatures in the vicinity of 10° to 15° C. The technique used for respiratory quotient determinations in the nembutal experiments was the best and most preferred technique, requiring the use of a glass chamber and a capillary lead tube for special respiratory quotient samples direct from the chamber. (See pages 14 and 20.) Aliquot samples were also analyzed for respiratory quotients, and it has been demonstrated that the lead tube method and the aliquoting method agree. By the use of the various equipments described previously we were able to measure (in addition to the respiration rate) the rectal temperature, the heart rate, and the heat production.

The combination of deep narcosis and the cold environment always produced a decrement in the various body functions, and thus it was possible to simulate a process of entering hibernation. The observations of the simulation of entering hibernation by the drugged marmot were far more complete than those made when it was entering normal hibernation, as we were able to control the conditions and to make the necessary preparations for all the various measurements. The marmot under the combined influence of the drug and the cold was entirely devoid of any voluntary movements. Muscular tone was also lacking, and the animal remained motionless as long as it was completely under the drug, there being only one or two cases where an animal partially recovered and then again succumbed to the

combined effects. The animals under nembutal, although their eyes were open, but lifeless, had no eye reflex. Also the animals did not urinate while under the influence of the drug.

As has already been mentioned, when the marmots were normally entering hibernation, respiratory quotients below 0.70 were sometimes found. When the marmots, under the influence of nembutal, were cooled to simulate entering hibernation, low respiratory quotients were found in all cases. These quotients, determined by gas analysis, frequently were under 0.50, and even a relatively large error in gas analysis could not possibly account for this low value. Moreover, the optimum conditions for gas analysis were obtained. That nembutal itself is not responsible for these low quotients was demonstrated by an experiment on No. 19, on December 2, 1936. This animal was injected with nembutal at 10.35 a.m., the dose being slightly

TABLE 28—*Effect of decreasing rectal temperature on apparent respiratory quotients of marmots under nembutal*

Marmot	Date	Rectal temperature ¹		Respiratory quotient ²		Heat production per $10\text{w}^{2/3}$ per 24 hrs.	
		Minimum	Rate of fall per hour	Maximum	Minimum	Maximum	Minimum
25	Dec. 4	10.6	1.8	0.62	0.44	750	250
18	" 7	15.1	2.4	.72	.64	260	80
3	" 18	23.4	2.3	.69	.62	270	90
1	May 22	16.5	2.3	.54	.44	180	33
2	" 24	12.9	2.6	.56	.51	210	33
	" 27	15.0	2.7	.57	.49	100	60

¹ About 37° C. before cooling.

² Assumed to be 0.70 before cooling.

under that required for complete narcosis, and placed in the refrigerator at 10° C. For several hours the animal was unconscious and, for the most part, motionless, but the rectal temperature was not significantly lowered. The metabolism was studied at various times during the day. The quotients were essentially 0.70, the lowest observed being 0.68. This is slightly, but not significantly, below that of fat and is much higher than quotients found with marmots entering hibernation or cooled under nembutal. It, therefore, is evident that these low quotients are associated with the process of entering hibernation or the rapid cooling of drugged animals.

Table 28 summarizes the data for the marmots under nembutal studied as they were rapidly cooled. The respiratory quotients of these animals varied from a maximum of 0.72 to a minimum of 0.44. No animal had a minimum quotient above 0.64.

The respiratory quotients and the rectal temperatures of No. 18 on December 7 have been plotted in figure 7 with reference to the time of day. This

chart shows that as the rectal temperature falls, there is simultaneously a fall in the respiratory quotient. As the rectal temperature ceases its descent, noted at approximately 7 p.m., the respiratory quotient also maintains temporarily a constant level at 0.65 but later tends to rise. The respiratory quotient, therefore, seems to be associated with the course of the rectal temperature.

From table 28 it will be noted that the rate of fall of the rectal temperature is very fast, being from 1.8° to 2.7° per hour. It will also be noted that the level of the metabolism is low, the maximum being 750 calories and the minimum as low as 33 calories per $10w^3$ per 24 hours.

The retention of carbon dioxide by the cold body fluids when the total production of carbon dioxide is at an extremely low level might readily result in a low respiratory quotient. To strengthen further such a theory is the fact that as the animal's body temperature becomes uniform at

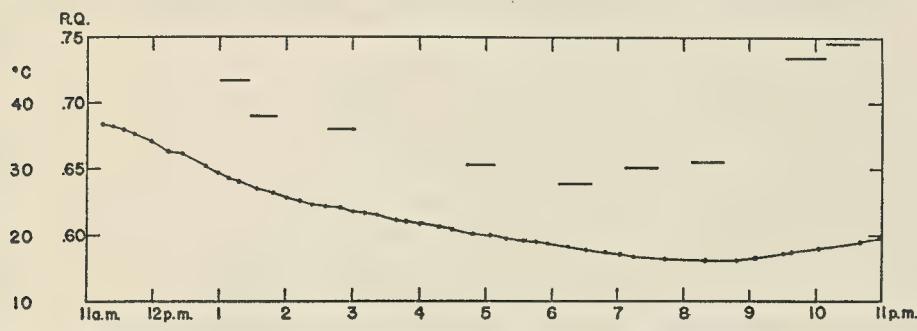


FIG. 7.—APPARENT RESPIRATORY QUOTIENTS AND RECTAL TEMPERATURE OF MARMOT 18 ON DECEMBER 7, 1936, COOLED, AND UNDER NEMBUTAL

Measured respiratory quotient (represented by dashes) shown to decrease simultaneously with decreases in rectal temperature (represented by connected dots).

the lower level the respiratory quotient tends to rise, that is, the body cells are approaching saturation with carbon dioxide for the prevailing temperature. It must be again emphasized that the true physiological respiratory quotient is the carbon-dioxide *production* divided by the oxygen consumption, but that the determination of the respiratory quotient is carried out by measuring the carbon-dioxide *elimination* and the oxygen consumption. Since at 10° carbon dioxide is thirty-one times as soluble as oxygen, it is obvious that of these two gases carbon dioxide is the one that would be materially affected by the cooling of the body.

As the fall in rectal temperature produces a low respiratory quotient provided the heat production is at a low level, a rise in the rectal temperature should result in a quotient above that of fat due to driving off some previously absorbed carbon dioxide which, when added to the true carbon-dioxide production, would result in a quotient above 0.70. It may be seen from figure 7 that at 9.30 p.m. and 10.07 p.m. the rectal temperature of marmot 18 was increasing and that the respiratory quotient at these times did rise above

0.70, namely, to 0.73 and 0.74. This is added proof of the contention that the physiological respiratory quotient is that of fat and that the low apparent respiratory quotients observed in the process of entering hibernation are due to the retention of carbon dioxide by the tissues of the animal.

On December 10, No. 18 (under nembutal) presented a different picture. The rectal temperature decreased slowly, falling but 9° in 14 hours, the metabolism averaged 150 calories per $10w^{\frac{1}{2}}$, and the respiratory quotient was that of fat. Thus with a *gradual* fall in body temperature the retention of carbon dioxide by the body represents but a relatively small part of the total carbon-dioxide production and does not measurably affect the respiratory quotient.

The respiratory quotient has been demonstrated to be low during the process of entering hibernation due to the fact that carbon dioxide is more soluble as the body temperature of the animal falls. This theory will not explain the low respiratory quotients found in the case of the normally hibernating marmot when at a steady state. It also will not explain those nembutal experiments where low respiratory quotients were found with the marmot in the steady state. However, in this steady state both when the marmot was under nembutal with a lowered rectal temperature and in the normal hibernating condition low quotients were not invariably found. It becomes obligatory, therefore, to establish just what the condition is that causes these low respiratory quotients with some marmots in the steady state of hibernation and not with others. It was also noted that when the respiratory quotient was low in the steady state, the heat production was usually at a very low level. Animal No. 24, under nembutal, was studied for a number of days. It was maintained for the most part at a constant rectal temperature level of 11°C. The metabolism was very low, approximately 30 calories per $10w^{\frac{1}{2}}$, and each day the respiratory quotient was below 0.70. Thus, on January 11 the quotient was 0.64, on January 12 it was 0.65, and again later that day 0.67, but the following morning it was down to 0.65. On January 14 it was 0.66 and later in the day was 0.67. From this finding of a constant low respiratory quotient from day to day one might be inclined to conclude that this is a physiological fact.

When the heat production is approximately 30 calories per $10w^{\frac{1}{2}}$ per 24 hours, the carbon-dioxide production must necessarily be extremely low, approximately 0.4 c.c. per minute. It is thus seen that a very small amount of carbon dioxide retained by the tissues would cause a great variation in the respiratory quotient.

The fact that on two days the respiratory quotient rose slightly as the day progressed challenged attention. Prior to January 14 the night ventilation of the chamber was purposely increased, to provide adequate ventilation in case the animal waked during the night and thus to avoid the death of the animal by asphyxiation. It was, therefore, necessary at the start of each day to stop the ventilation and allow the carbon-dioxide percentage in the air to increase to about 0.7, thus enabling an accurate analysis of the air.

The first measurements following an experiment with the slow ventilation rate maintained overnight resulted in finding respiratory quotients of 0.69. Later in the day with this slow ventilation continued a series of quotients of 0.70 were found. In fact, with the ventilation rate at this low level, the respiratory quotient was constantly found to be that of fat thereafter. It is thus necessary for the animal to come to equilibrium with the carbon dioxide in the surrounding air, in addition to having its fluids saturated at a given temperature. When the carbon-dioxide production is low, it is conceivable that the carbon-dioxide equilibrium in the body fluids will be different if the carbon-dioxide content of the atmosphere is maintained at practically that of outdoor air, 0.030 per cent, from that when it is maintained at nearer 0.7 per cent, as would be the case for respiratory exchange measurements.

With a normal hibernating marmot, M, in the series in August, 1935, fast ventilation prior to determining the metabolism invariably resulted in finding of low respiratory quotients down to 0.60. In the experiment when the ventilation was maintained at the slow rate, thus keeping the carbon-dioxide percentage at the optimum level of approximately 0.7, the respiratory quotients were that of fat. Specific proof that the fast ventilation was the cause of the finding of low respiratory quotients is shown by the fact that after the respiratory quotient level of 0.70 was established on August 3 with animal M, the ventilation was increased to a rapid rate and following this low quotients of 0.64 and 0.63 were again found. The heat production at this time was also at a very low level of 25 calories per $10w^{\frac{1}{2}}$. Thus we have additional proof that a long sojourn in the environment at which the animal is to be measured is necessary to obtain true respiratory quotients. The experiments on marmot No. 26 (after January 27) in which this equilibrium requirement was observed resulted in a persistent finding of respiratory quotients of fat (*i.e.*, 0.70).

One of the important outcomes of this research, particularly the nembutal series, is an explanation of the hitherto unexplainably low respiratory quotients reported by the Nutrition Laboratory in the case of the python.⁹ At a temperature of 18° the python showed consistently respiratory quotients of essentially 0.60, whereas at a temperature of about 30° the respiratory quotient was invariably that of fat. The influence of the solubility of carbon dioxide in body fluids was clearly recognized and extensively discussed by Kayser¹⁰ who furthermore, based upon experiments in which the carbon dioxide of the environmental air was raised to over 40 per cent, stressed the important rôle that the change in the alkaline reserve may have. In these studies with the marmot it is clear we have to deal with two separate factors that may obscure the true respiratory quotient, the first being the variations in solubility of carbon dioxide in the tissues due to changes in temperature

⁹ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 425, 1932, p. 411.

¹⁰ Dontcheff, L., and C. Kayser, Compt. Rend. Soc. de Biol., 1934, **118**, p. 81; *ibid.*, 1936, **121**, p. 446.

of the animal. This may be likewise supplemented by changes in the alkaline reserve. The second factor is the length of time required for the tissues to reach carbon-dioxide equilibrium when the percentage content of carbon dioxide in the environmental air ranges from 0.03 to approximately 0.7. As the python has a metabolism but one-third that of the marmot in hibernation, its adjustment to equilibrium will take a much longer time, indeed longer than the 8 hours usually covering our period of measurement. Our friend, Dr. Charles Kayser, of the Institute of Physiology, of the Faculty of Medicine of the University of Strasbourg, has fully convinced us that his criticisms of the low respiratory quotients reported for the python are sound. This study of the marmot was well under way before we had learned of the findings of Kayser, but we approached the problem from a somewhat different standpoint inasmuch as our use of nembutal was particularly striking in its effect. Thus the two researches may be stated to confirm each other completely, and as a result it is quite impossible to consider any quotients below 0.70 in the hibernating or the cold-blooded animal as being anything other than artifacts, for the true physiological quotient will not significantly vary from that of fat.

RESPIRATION RATE

In establishing the true respiratory quotient of the hibernating marmot strikingly low respiration rates as the animal entered hibernation were repeatedly found. Studies of the minimum respiration rate of the marmot in hibernation, the variability and the range in rates were made by ocular observations. One of the earliest series was made on marmot D on December 23, 1932. This animal, which had been overnight in a cold garage at an environmental temperature of 5°, was brought into the laboratory at a constant room temperature of 24°, and the respiration rate and rectal temperature were determined throughout the day. The rectal temperature started at 6.5° at 9.15 a.m. and rose gradually throughout the day to 22.0° C. at 5.20 p.m. The respiration rate followed an extraordinary course in that it was highest in the morning, 8 per minute, and descended throughout the day, reaching a minimum of 0.4 respiration per minute in the late afternoon. The decrease in the morning was rapid, the rate falling in hardly one hour from 8 to 6 per minute and in ten minutes more to 4 per minute. The animal showed no signs of waking when it was returned to the garage at 5.20 p.m.

On another day this same marmot confirmed this picture, but in a less striking way. At a constant environmental temperature of 15° the respiration rate underwent a reasonably uniform transition from an initial rate of 2 and 3 per minute at 11 a.m. to 1 per minute in the latter part of the afternoon, during which time the rectal temperature rose from 9.7° to 13° C. The actual rate at the end of the experiment was considerably higher in this case than the rate noted in the other experiment with D, that is, 1 is two and one-half times as high as 0.4, although the environmental temperature in the case of the respiration rate of 0.4 per minute was 24° and in the case where the

respiration fell only to 1 it was 15.8°C. One might expect with a high rectal temperature a higher metabolism and hence an increased respiratory activity to remove the carbon dioxide. That this is not necessarily always the case will be shown subsequently. (See page 170.)

Other scattered observations have been made in connection with our various studies, in which it has also been noted that the rectal temperature has risen during the day and the respiration rate has shown a slight decrease. Thus, on January 28, 1937, No. 26 had a rectal temperature in the morning nearly constant at 3.5° C. and a respiration rate of 1 per minute. During the course of the afternoon the rectal temperature rose from 3.3° to 9.1° at 5.24 p.m., during which time the respiration rate varied but finally reached 0.4 per minute. Most of the other extremely low respiration rates, under 1 per minute, were accompanied by rectal temperatures, when measured, of 11° or below, so that in general the low respiration rate is associated with the low rectal temperature.

The material presented in table 20 (page 101) represents not simply marmots in hibernation but, as can be seen from the rectal temperatures, animals that were at times awake. This table shows a number of instances where the respiration rates approximate the minimum basal respiration rate of the non-hibernating marmot, *i.e.*, in the neighborhood of 25 to 30 per minute. On the other hand, there are instances where rectal temperatures in the vicinity of 36° are accompanied by respiration rates three or four times this, notably in the case of K in the latter part of April.

If a certain body temperature level is selected and the respiration rates at this level on different days are inspected, it is found that there is no uniformity in the rates. Thus, with marmot I at a body temperature of 11° respiration rates were found ranging from as high as 13 on February 12 to as low as 0.5 on March 31 and April 1. At the same body temperature K's rates ranged from 18 per minute on April 14 to 1 per minute on March 31. No. 26 (see table 29) also at approximately 11° had rates from 1 to 0.4 per minute. Thus it is seen that the respiration rate is not constant at a given rectal temperature.

When slow respiration rates occur, there is at times a long pause between one respiration and the next, as was also noted by Dubois¹¹ and others. Marmot W on March 12, for example, had one respiration in 0.18 minute, and the second respiration did not occur until 3.97 minutes later. The same animal on March 24 had one respiration in one minute, and the second respiration occurred 1.79 minutes later. Marmot U on April 2 had one respiration in 0.63 minute, a second respiration 3.01 minutes later, and a third respiration in 0.88 minute after this, again another picture of variability in respiration rate.

As the respiration rate of the normal non-hibernating marmot is about 25 per minute and those rates less than 25 per minute may be considered to be indicative of incipient or deep hibernation, perhaps the most obvious deduc-

¹¹ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, pp. 46 et seq.

TABLE 29—Respiration rate and heart rate during normal hibernation
(Marmots 26 and 19)

Marmot, date, and time	Temperature		Rate per minute		Marmot, date, and time	Temperature		Rate per minute	
	Envi- ron- mental	Rectal	Respi- ration	Heart		Envi- ron- mental	Rectal	Respi- ration	Heart
1937	°C.	°C.			1937	°C.	°C.		
26					26				
Jan. 27					Feb. 10				
2.07 p.m.	4.2	5.9	0.7		8.10 a.m.	9.4	10.1	.	5
3.31 p.m.	.	.	0.6		8.34 a.m.	9.3	10.1	.	5
4.56 p.m.	3.1	5.2	0.7		9.01 a.m.	9.8	10.2	0.8	5
11.35 p.m.	2.6	4.0	0.9		9.28 a.m.	10.2	10.5	.	5
Jan. 28					9.43 a.m.				6
8.00 a.m.	2.1	3.8	1		10.04 a.m.			0.8	.
8.52 a.m.	2.2	3.7	1		10.29 a.m.			.	5
9.27 a.m.	2.2	3.7	1		10.41 a.m.			.	6
11.38 a.m.	2.2	3.6	1		11.01 a.m.	10.2	10.4	.	5
12.03 p.m.	4.0	3.3	1		11.11 a.m.			.	5
12.50 p.m.	11.9	5.4	2		11.25 a.m.			.	5
2.25 p.m.	9.3	7.5	1		11.31 a.m.	10.6	10.6	.	5
3.32 p.m.	10.3	8.2	0.3		11.52 a.m.	10.3	10.6	.	5
5.24 p.m.	.	9.1	0.4		11.57 a.m.			1	.
Jan. 29					1.14 p.m.	10.0	10.7	.	9
8.20 a.m.	11.0	11.1	1		1.48 p.m.			.	5
9.32 a.m.	10.6	10.9	1		1.58 p.m.			.	6
Jan. 30					2.30 p.m.	11.2	11.2	.	6
7.35 a.m.	.	11.0	0.4		3.12 p.m.	11.2	11.0	0.6	.
Feb. 4					Feb. 15				
9.05 a.m.	.	.	4	19	4.55 p.m.	9.2	.	0.7	7
9.40 a.m.	1.7	5.6	.	20	9.45 p.m.	10.2	10.7	.	6
9.47 a.m.	1.8	5.5	4	20	Feb. 16				
10.07 a.m.	1.8	5.5	4	21	8.23 a.m.	10.4	11.2	.	5
10.27 a.m.	1.8	5.6	4	21	8.57 a.m.	10.8	11.2	0.8	4
10.47 a.m.	1.9	5.6	4	19	9.32 a.m.	10.8	11.3	.	5
11.17 a.m.	1.8	5.7	4	37	10.22 a.m.	10.2	11.2	1	5
11.48 a.m.	.	5.7	.	22	10.38 a.m.	10.7	11.2	.	5
11.49 a.m.	1.8	5.9	.	24	11.07 a.m.	10.8	11.2	.	4
12.20 p.m.	.	.	.	14	11.27 a.m.	10.2	11.4	.	5
12.25 p.m.	.	.	.	20	11.52 a.m.	9.0	11.4	.	5
12.26 p.m.	.	.	.	19	12.04 p.m.	8.2	.	2	5
12.30 p.m.	.	.	3	18	12.37 p.m.	7.1	10.7	.	4
12.46 p.m.	8.0	6.4	2	10	12.52 p.m.	6.6	10.4	.	4
1.02 p.m.	11.3	6.6	.	15	1.27 p.m.	5.7	9.8	.	4
1.03 p.m.	.	.	2	13	2.14 p.m.	5.0	9.1	.	4
1.16 p.m.	11.7	7.3	.	19	2.41 p.m.	4.7	8.5	0.6	4
1.23 p.m.	.	7.6	3	20	3.12 p.m.	4.7	8.0	.	4
1.32 p.m.	11.7	7.8	.	26	4.01 p.m.	4.1	7.6	.	5
Feb. 9					4.19 p.m.	4.0	7.3	.	4
4.09 p.m.	11.7	11.6	0.6	5					
4.41 p.m.	11.0	11.2	.	5	19				
4.53 p.m.	11.0	11.2	.	6	Feb. 13				
5.04 p.m.	.	.	.	5	12.10 p.m.	.		.	20
5.44 p.m.	10.0	10.7	0.5	5	7.30 p.m.	.		.	5
6.23 p.m.	.	.	0.3	.	11.55 p.m.	.		.	5
6.35 p.m.	10.8	11.2	.	4	Feb. 14				
7.03 p.m.	10.6	11.0	.	5	8.20 a.m.	.		10.7	7
7.12 p.m.	.	.	.	4	12.30 p.m.	.		10.4	6
8.35 p.m.	.	.	.	5	6.20 p.m.	.		10.6	6
9.30 p.m.	.	.	.	4					

tion from these observations is that the respiration rate of the marmot during hibernation is extremely labile. The differing degrees of somnolence frequently found with these marmots might affect the respiration rate. It is possible for an animal to retain a respiration rate of essentially 1 per minute throughout the entire day, as shown by No. 26 on January 28. Indeed, most of the values appearing in table 29 (see page 136) are 1 per minute or under. At this time, however, we are especially interested in noting the minimum respiration rate in deep hibernation. The details shown in table 30 for No. 1 are representative of the picture with eleven other marmots studied simultaneously. From the data for No. 1 alone one can conclude that the respiration rate during deep hibernation may be as low as 0.3 per minute. The lowest rate found with K (see table 20, page 101) was 0.2 per minute and with marmot I, 0.2 per minute, indicating 1 respiration in 5 minutes. Many other marmots studied gave essentially this same picture. It is, therefore, clear that the marmot may have as low a respiration rate as 1 in 4 or 5 minutes. This rate probably represents deepest hibernation, but again the question arises as to what are the criteria as to the depth of hibernation.

This variability of respiration rate is so closely associated with the depth of hibernation, the rectal temperature, and the fact that many of these animals had intermittent periods of waking up and subsequent returning to hibernation that a final interpretation of these results is unjustifiable. The hibernating marmot may descend to a respiratory level where there is but one respiration in 5 minutes, this being 0.2 per minute or hardly one one-hundredth of the minimum value ascribable to the non-hibernating marmot at its normal body temperature of not far from 37° C.

Although it has been recognized that the respiration rate is not invariably a sharp criterion as to the depth of hibernation, nevertheless an examination of the data in table 30 for No. 1 shows that the level prior to the beginning of hibernation is usually above 30. From the designations A (awake), S (sleeping), D (drowsy), and H (hibernating), one can see immediately a certain association between these different conditions and the respiration rate. The table shows the fluctuations in the respiration rate of the marmot as time goes on, although these were not particularly marked in the first month or six weeks. There was then a long period of hibernation interwoven with the characteristic waking up for a day or two, and finally the marmot was definitely awake at the end of the observations, the last two being on April 20 and 29. In general, the temperature of the garage varied from 3.3° on one day to as high as about 14°, remaining for the greater part of the time in the neighborhood of 10°.

The rapidity of change of these animals when passing from one state to the other is remarkable. Using for the moment the respiration rate as an index of the depth of hibernation, along with the designated condition in table 30, we find a sudden change with No. 1 on March 25 to 26. On the first of these days the animal was in the hibernating state and had a respiration rate of 1 and on the next day the animal was awake, with a respiration rate

TABLE 30—*Fluctuations in respiration rates of marmot No. 1 over a period of 5 months*

Date	Condition ¹	Rate per minute	Date	Condition ¹	Rate per minute
<i>1936</i>					
Dec. 3	A	40	Feb. 9	H	1
" 4	A	48	" 10	A-D	33
" 5	A	24	" 11	H	3
" 7	A	43	" 12	H	1
" 8	A	50	" 13	H	6
" 9	A	22	" 15	H	1
" 10	A	26	" 16	H	1
" 11	A	53	" 17	A	71
" 12	A	38	" 18	H	2
" 13		42	" 19	H	1
" 14	A	45	" 20	H	2
" 15	A	33	" 23	H	0.5
" 16	A	33	" 24	H	0.5
" 17	S	31	" 25	H	0.8
" 18	D	9	" 26	H	0.8
" 19	DA	26	" 27	H	0.3
" 21	AA	32	March 1	H	0.8
" 22	S	17	" 2	H	1
" 23	SA	36	" 3	H	0.5
" 24	H	0.7	" 4	H	1
" 28	A	53	" 5	H	1
" 29	A	56	" 6	H	2
" 30	H	3	" 8	H	2
" 31	H	1	" 9	D	10
<i>1937</i>					
Jan. 2	H	1	" 12	H	0.9
" 4	A	42	" 13	H	3
" 5	H	0.5	" 15	H	2
" 6	H	2	" 16	H	4
" 7	DH	31	" 17	H	2
" 8	H	0.4	" 18	H	2
" 9	H	3	" 19	A	71
" 11	A	50	" 20	H	1
" 12	H	2	" 22	H	1
" 13	H	0.4	" 23	H	3
" 14	H	2	" 24	D	8
" 15	DH	0.5	" 25	H	1
" 16	DH	0.5	" 26	A	36
" 18	H	0.4	" 27	D	19
" 19	H	0.9	" 29	H	3
" 20	H	8	" 30	H	4
" 21	A	48	" 31	H	2
" 22	H	3	April 1	A	71
" 23	H	0.3	" 2	A	63
" 25	H	0.8	" 3	H	1
" 26	H	0.9	" 5	H	3
" 27	H	1	" 6	H	1
" 28	H	2	" 7	A	56
" 29	H	0.5	" 8	A	48
" 30	H	2	" 9	H	1
Feb. 1	A	45	" 12	H	2
" 2	A	50	" 13	H	1
" 3	A	48	" 14	H	1
" 4	D	7	" 15	H	1
" 5	H	2	" 16	A	71
" 6	A	36	" 20	H	71
" 8	H	0.5	" 29	A	91

¹ A = awake; S = sleeping; D = drowsy; H = hibernating.

of 36. The reverse condition is likewise frequently found, where the animal may be awake with a high respiration rate and in 24 hours have a very low rate. Thus on January 4 No. 1 was awake with a rate of 42 per minute and on January 5 it was hibernating with a rate of 0.5 per minute.

The large mass of data collected with other animals at this same time would require too great space for detailed publication. As a typical illustration of the general picture during the period of deepest hibernation eight days of observations, March 17 to 25, have been selected for publication. (See table 31.) This table gives further evidence that where the condition is

TABLE 31—*Typical respiration rates of marmots¹ during period of deepest hibernation*

Date	No. 1		No. 2		No. 4		No. 6		No. 9		No. 11	
	Condition ²	Rate per minute										
<i>1937</i>												
March 17	H	2	H	0.5	H	0.6	H	2	H	4	H	2
" 18	H	2	H	1	H	0.4	H	0.5	H	2	H	0.5
" 19	A	71	H	0.5	H	0.4	H	3	H	2	H	2
" 20	H	1	H	1	H	0.7	A	90	H	0.5	A	37
" 22	H	1	H	1	H	0.6	H	0.5	H	3	H	0.9
" 23	H	3	H	1	A	56	H	2	H	5	H	0.9
" 24	D	8	H	1	H	1	H	1	A	23	H	0.6
" 25	H	1	H	0.3	H	0.5	H	0.5	H	5	H	0.3
<i>1937</i>												
Date	No. 12		No. 19		No. 22		No. 23		No. 24		No. 27	
	Condition ²	Rate per minute										
<i>1937</i>												
March 17	H	0.5	H	0.6	D	20	H	0.6	A	55	H	0.5
" 18	H	2	H	0.5	D	13	H	0.2	A	62	H	1
" 19	H	1	.	0.6	D-H	3	.	0.5	D	17	H	0.8
" 20	H	0.6	H	1	H	0.9	H	0.7	H	2	H	0.8
" 22	H	0.4	H	0.5	H	3	H	2	H	9	H	0.3
" 23	H	0.5	H	0.6	D	7	H	0.4	A	71	H	0.5
" 24	A-D	48	H	0.5	H	9	H	1	H	1	D	4
" 25	H	0.7	A	83	A	50	H	0.4	H	7	H	0.6

¹ Living at an environmental temperature of about 7° C.

² A = awake; D = drowsy; H = hibernating.

noted as "H" respiration rates of over 9 do not occur, and in general the rate is much lower. The instances where "A" appears are never accompanied by a low respiration rate. Thus the respiration rate is a crude index of the condition of the animal. The respiration rates in this series were all counted when the marmots were in their usual cages out in the cold garage. Although every effort was made to obtain these counts most accurately, it was recognized that the low rates and at times the rather feeble respiratory movements taxed the observational powers of the observer too greatly to insure absolutely accurate counts.

In another series, a marmot in normal hibernation was placed in a glass

chamber inside an electric refrigerator provided with a glass door. (See plate 2, page 12.) This could be well illuminated, and a trained observer could be easily certain of respiratory counts. Emphasis at this time was laid upon marmots in deep hibernation, with no particular interest in those awake or in the intermediary phases. Data were thus obtained for No. 26, studied intermittently over a period from January 27 to February 16 (see table 29, page 136), and for the greater part of the time the animal was in deep hibernation. These observations show that the respiration rate during hibernation usually is well under 2 per minute and actually with this animal went as low as 0.3, thus confirming the general finding with the entire group of marmots that the respiration rate may descend to as low as 0.2 or 0.3 and that the normal rate in hibernation hovers around 1 per minute. In this particular study with No. 26 the rectal temperature was studied along with the respiration rate, and from February 4 on the heart rate was also studied. Further discussion of the observations on this marmot, including the measurements of the respiratory exchange, will be given later. (See pages 167, 170, and 177.)

In studying the marmot hibernating under the influence of nembutal, data on the respiration rate were also obtained. Frequently respiratory failure occurred with the narcotized animal before its respiration rate reached the low levels noted with the normally hibernating animal. Rates of 1 per minute were noted, but usually they were more nearly 4 or 5 per minute. The minimum respiration rates were usually noted at a rectal temperature of about 11° or 12° C. Of six marmots under the influence of nembutal only one, No. 25, had a rate of less than 1 per minute. This animal in two observations had rates of 0.5 and 0.6 respiration per minute at a rectal temperature of approximately 10° C.

As has already been pointed out, the respiration rate of the marmot is variable under relatively uniform conditions of environmental and rectal temperature. The depth of respiration also is by no means always uniform, and the respiratory rhythm is not uniform. It is not infrequent to note perhaps one deep breath followed by several shallow respirations. Thus the rate of respiration is not an accurate index of the metabolic activity. The per minute volume, calculated from the rate and the depth of respiration, probably would have more physiological significance but unfortunately data regarding the depth are not available. Most of the respiration rates were obtained by visual observation, but in the case of some of the marmots under nembutal the respirations were recorded graphically with the use of a tambour and a light balloon (see figure 1, page 14). The variability in the depth and rate is shown for No. 1 on May 26 in figure 8.

General conclusion with regard to the respiration rate during hibernation. Some of the great variability in the respiration rate of the marmot noticed under conditions that are seemingly static is accounted for by the extreme lability in the vital activity of this animal, its inclination to go into hibernation step by step and, having once entered, to pass rapidly from hibernation

to awake and back again within a few hours accompanied by (as will be seen later) profound changes in the gaseous metabolism and the necessity for complete ventilation of the lungs. The marmot can exist over a considerable period of time with a respiration rate corresponding to but 1 respiration in 5 minutes. In general the respiration rate during hibernation is a little less than 1 per minute, as compared with the normal, quiescent, non-hibernating basal rate of not far from 25 to 30 per minute. Excitation may increase the rate to 100 respirations per minute or more. There is evidence of a paradoxical slowing of the respiration rate with an increase in rectal temperature, especially when the latter ranges from 5° to 15° or even 20° C. This unusual relation between rectal temperature and respiration rate emphasizes a distinct gap in the research, in that the respiratory volume per minute could not be determined, at least without operative procedures. The lability in the respiration rate as the marmot goes into hibernation, during hibernation, as it comes out, and when normally awake is further evidence of the general picture noted with these animals, namely, of extreme vari-



FIG. 8—GRAPHIC RECORD OF RESPIRATION RATE OF MARMOT 1, ON MAY 26, 1937, COOLED AND UNDER NEMBUTAL

Time marker above shows minutes. Record shows irregularity characteristic of marmots both under nembutal and normally hibernating.

ability in metabolic activity. The lowest respiration rate in hibernation, under the influence of nembutal, is much higher than rates found in normal hibernation. In the latter condition the rate may descend to 1 in 5 minutes, but under nembutal the minimum rate is 1 in 2 minutes, and as the average minimum rate is nearer 4 or 5 per minute under nembutal, it can be seen that this does not perfectly simulate the normal hibernating condition. It has been pointed out that in the normal condition the respiration rate may be taken as a rough index of the depth of hibernation. Fortunately later data permit comparisons directly between the heart rate, respiration rate, rectal temperature, and the important factor, gaseous metabolism.

HEART RATE

Because of the close association of heart rate and heat production noted with humans and animals, it was of particular interest to measure the heart rate of these marmots when in the unique hibernating condition. Since the animals are so easily stimulated to waken, the attachment of a stethoscope to a hibernating marmot without disturbing it is an impossibility. By the use of a modification of the Boas cardiotachometer (see page 13) devel-

oped to measure the elephant's heart rate, we were able late in our research to secure data on this function with a few animals. Marmot M, the first animal studied, was measured in October 1935 after being in hibernation several months. The animal was kept in a refrigerator in which the temperature was from 4° to 11° C. On the 3rd of October, the electrodes were attached and intermittent measures over a period of two hours averaged 12 beats per minute. Because the electrodes required better contact, it was necessary to reattach them more firmly. This act evidently stirred up the animal, as the heart rate jumped to 40 per minute. Three hours later the rate had settled down to 7 per minute, the lowest level thus far noted. The rectal temperature determined at this time was 9.3° C. Measures of the heart rate within the next 10 minutes gave higher rates of 17 and 13 due to the stimulation of taking the rectal temperature. On October 4 the animal gave two rates of 21 and 15 at 9.35 and 9.37 a.m., with an environmental temperature of 6°. By afternoon the rate had fallen to 8 per minute. The animal was left overnight with the contacts on, and the rate at 9.10 a.m. on the next day averaged 8.4 per minute. On October 9 the animal remained from 11 a.m. to 5 p.m. at a refrigerator temperature of 6° and the heart rate varied from 10 to as high as 56 and ultimately went up to 100, at which time the rectal temperature was found to be 8.2°. The next morning the animal was found with the electrodes removed. Apparently it waked up completely during the night but had gone into hibernation again, for after the electrodes were reattached at 11.39 a.m. the rate was 18 per minute. That afternoon the rate had fallen to 8, where it remained until 8.12 p.m. The next morning the level of 13 per minute was observed, with 5 respirations being noted in 2.3 minutes. At noon the rate was still 14 per minute but in the afternoon from 3.18 to 5.08 p.m. it averaged 8 per minute. The rectal temperature at this time was 6.4° C. On October 14th the animal was definitely awake, but the electrodes had not been disturbed and the heart rate was 64 per minute at 8.40 a.m. and 154 at 9.37 a.m. The next morning at 11.30 a.m. the rate was down to 8 where it stayed for the greater part of the day, rising on two occasions to 15 and 16. On October 16 the rate of 9 per minute was maintained throughout the entire day. The rectal temperature was 5.8° with a respiration rate of 1 to 2 per minute. On October 17 it was noted that the heart impulse was very weak, the rate being about 8, where it held throughout the entire day. Then the refrigerator was disconnected by mistake so it warmed up during the night and the next morning, October 18, with a rectal temperature of 32.9° C., the animal had a heart rate of 134 per minute. On the 19th, with the refrigerator at 6° the heart rate was again down to 9 or 10, where it stayed all day, with a rectal temperature of 8.0° C.

For this animal, therefore, it can be seen that the lowest value was not far from 7 to 8 beats with a rectal temperature of approximately 5° or 6° C. On the other hand, when the animal was awake, as on October 18, with a

rectal temperature of 32.9° C. the value was 134, and on October 14 with the animal awake it was 64 and 154, but the rectal temperature was not taken.

With animal No. 19 (see table 29) observations on two days gave an essentially constant level of 5 to 7 beats per minute measured at various times during the day. The rectal temperature of this animal was 10° C.

The most complete series of measures accompanied by other observations was obtained on animal No. 26. On February 4 the rate was very constant, averaging 20 per minute, while the rectal temperature was 5.7° C., and when the environment was warmed, causing a rise in the rectal temperature, the heart rate decreased to 10 beats per minute. The warming stimulated the animal to waken so that the rate subsequently rose. Previous to February 9 the electrodes were again attached, and measures on this day varied only from 4 to 6 beats per minute. The rectal temperature was 11° C. The following day the range of the heart rate was 5 to 9 per minute, but averaged close to 5. Later in the day the animal was intentionally wakened.

The animal returned to hibernation within a day or two and measures on February 15, when its rectal temperature was approximately 11° C., showed rates of 6 to 7 per minute.

The following day, February 16, frequent measures were made. There was a range in temperature from 11.4° to 7.3° C. On this day over a period of 8 hours, with a body temperature variation of more than 4° C., the rate remained remarkably constant, being only from 4 to 5 beats per minute.

THE NON-REGULARITY OF THE HEART RATE IN DEEP HIBERNATION

It was commonly noted that these heart beats were not regularly separated or timed. Unfortunately no photographed records or electrocardiograms were made, as we were not in a position to make photographic studies with the pressure of the work going on. On one day with animal 26 we did, however, measure the time required for each of 21 consecutive beats, using a stopwatch, and these are given in table 32. Although it is true that the average was very close to 5 per minute, it is of interest to note that the rate of the individual beats varied from 12.5 to 3.4 per minute.

Animal No. 24 was maintained at a low cell temperature under nembutal for a period of 12 days. The animal was measured over a period of 11 days, and the heart rate varied only between 15 and 21 in this entire series. During the entire time the animal was not seen to move. One eye, which was visible, remained partially opened and the position of the animal against the wall of the glass chamber was constant from day to day.

It is self-evident that the minimum heart rate does not occur at the lowest rectal temperature, and the correlation of the heart rate with other functions will be discussed later. The heart beats are not uniformly spaced, but a count of several beats gives a reliable index of the average rate. The minimum heart rate of the marmot in deep hibernation established by these

measures is 4 to 5 beats per minute, fully confirming the rate found by Dubois¹² as early as 1896.

TABLE 32—*Variations in heart rate of the hibernating marmot*
(Marmot 26, February 9, 1937)

Count	Time min.	Rate per minute
0	0.00	
1	.19	5.3
2	.30	9.1
3	.55	4.0
4	.67	8.3
5	.85	5.6
6	1.12	3.7
7	1.37	4.0
8	1.51	7.1
9	1.77	3.8
10	1.94	5.9
11	2.13	5.3
12	2.38	4.0
13	2.66	3.6
14	2.74	12.5
15	2.98	4.2
16	3.22	4.2
17	3.32	10.0
18	3.61	3.4
19	3.80	5.3
20	3.98	5.6
21	4.20	4.5

RECTAL TEMPERATURE

In the completely inert condition of these animals, the rectal temperature measurement becomes an important feature of the study. Unfortunately the extreme sensitivity of these animals to external stimuli interfered with and prohibited many such measurements, as the insertion of the thermometer was usually sufficient stimulus to wake the animal and have it pass out of the hibernating state, thus defeating our ends. Out of the relatively large population of animals, there are data on a sufficient number to give a fairly complete picture of the rectal temperature during the process of hibernation.

Comment has already been made on the transition of the rectal temperature as the animals go into hibernation, but since this phenomenon, only rarely noted, involved several days and the insertion of the thermometer would tend to stop or inhibit the passing into hibernation, the data on this point are not numerous. It was only with the insertion of the thermojunction that these studies could be made satisfactorily, but here also a certain degree of stimulus due to inserting the thermometer is involved, and interested as we were in the transition of going into hibernation, it was practically impossible to insert a thermometer in an animal fully awake and

¹² Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, p. 47.

maintain it there intact during the process of transition into the hibernating state. The best we could do, with one exception, was to find animals in a semi-stuporous condition, insert the thermo-junction, and hope the stimuli would not wake the marmot and would allow it to pass definitely into deep hibernation. The one exception has been referred to in the discussion of entering hibernation. The difficulty encountered in obtaining the hibernating condition at will, using the rectal temperature in this case for the criteria of hibernation, might be overcome by the use of another animal, such as the bat. Eisentraut¹³ has shown that even in summer the bat goes through a period of lethargy during the day accompanied by a lower body temperature, followed by a wakeful period at night. This process of lowering the temperature takes place daily. Thus the bat might be a splendid subject for this study.

Our studies consist of two distinct types of measurement, one with a mercury thermometer, and the other with the thermo-junction. In all cases, to insure that the galvanometer constant had not changed, the thermo-junction was carefully controlled by frequent calibrations of the junction temperature against an accurate mercurial thermometer. When the rectal temperatures were very low, the temperature of the reference thermometer was usually lowered, so as to have the galvanometer deflection at a moderately small magnitude to eliminate large errors of computation in applying a galvanometer constant per millimeter deflection to a large deflection. This was especially important when we were establishing the minimum temperature at which these animals could live.

In a study of the rectal temperature of the hibernating marmot there are several points to be considered. First, is the marmot in deep hibernation a true cold-blooded animal, in which case the body temperature would be very closely associated with the environmental temperature, that is, is there anything like a reasonably constant differential between the temperature of the animal in deep hibernation and the environmental temperature? Second, is the temperature of the hibernating marmot definitely below that of the environment, a situation demonstrated with cold-blooded animals? Third, what is the reaction of the rectal temperature to changes in the environmental temperature? Fourth, is there evidence as to the rapidity of such reactions to changes in environmental temperature? Fifth, what is the minimum temperature to which the body temperature can be lowered?

Ideal conditions in this study would have been to have the animal at a room temperature without change, over a considerable period of time. This was found to be the case relatively infrequently, for temperature regulation of the cold garage, where the animals were kept for most of the time, was not perfect. Table 20 (see page 101) shows daily measures of the rectal and room temperatures on two animals kept in the garage. In the instances where the rectal temperature is high the animal is either awake or in

¹³ Eisentraut. M., Zeitschr. Morph. u. Ökol. Tiere, 1934, 29, p. 231.

a transition condition. If the data from March 22 to April 14 are specifically considered, omitting the rectal temperatures of 25° C. and above, it is very evident that the rectal temperatures of both animals have the same general trend and, in addition, they are definitely related to the room temperature. On March 31 with the room temperature at 12°, the rectal temperatures are 11.5° and 11.9° C. At the higher room temperature of 16° on April 3, the body temperatures are also higher, being 15.5° and 17.0° C. From these data it is apparent that there is a relation of the rectal temperature to the environment, but since these measures were not conducted under static conditions, the exact relation cannot be determined from this table.

In some instances where the room temperature remained reasonably constant, and at some time previous a thermo-junction had been inserted, so that any possible effect of this stimulus had passed, valid measures were obtained in studying the exact relation of the rectal temperature to the environment. The differential between the room temperature and the rectal temperature, under the reasonably stable conditions that occurred on February 6, 1933, with animal G (when the room temperature ranged only from 0.8° to 2.5° C., and the rectal temperature ranged only from 3.4° to 6.1° C.) was not far from 3° C. Two other animals in the same room at substantially the same environmental temperature showed a differential throughout this period of from 1.0° to 3.5° C. At a room temperature of approximately 8° the differential was usually about 1° C. At environmental temperatures of 18°, the differential was about 1.3° C. Therefore, within the limits of about +4° to 18°, the hibernating animal follows, in general, the temperature of the environment, especially when it has been exposed to this for a considerable period of time, and usually the rectal temperature is from 1° to 3° above the temperature of the environment.

The next salient point is to establish if the marmot can be considered ever to have a temperature below the environment. Referring again to table 20, one sees that there is apparently this possibility, but from the nature of the garage (an outdoor building subject to the diurnal fluctuations in temperature with the regularly occurring rise in the forenoon from a minimum cold during the latter part of the night) the element of a fluctuation in environmental temperature was introduced. The protocols show frequently that beginning with the first observations in the morning the animal had a temperature below the room temperature. This was found in a good many instances. The room temperature was observed on a mercury thermometer in the cage at the time the rectal temperature was taken. Fortunately we had a recording thermometer in the garage, and when reference was made to these records it was found that the animal's rectal temperature was never below the environmental temperature to which it had been exposed for the past several hours. When the environment was warmed, the room temperature reacted more rapidly than did the body to the change in temperature. Hence it occasionally happened that only at the end of the day

did the rectal temperature exceed that of the room. We have no indications, therefore, of the rectal temperature of these animals being lower than the environment when conditions of stability had preceded the observation. In this way, therefore, the marmot is definitely not a true cold-blooded animal, for at a constant environmental temperature the body temperature of the latter is invariably below the environmental temperature.

REACTION TO SUDDEN CHANGES IN ENVIRONMENT

In two instances the animals were brought from the garage at a temperature of 5° C. into the laboratory at a temperature of 14° and 24° C., respectively. When the animal was transferred (in the forenoon) from a temperature of 5°, its rectal temperature was 6.5° C. After exposure at 14° the rectal temperature rose to 13.4° at 4.36 p.m., when the observations ceased. Actually it had not reached the temperature of the environment. (Animal D, December 21, 1932.) In another case when the animal was transferred suddenly to a room at 24.5° the animal at 9.15 a.m. had a temperature of 6.5° and in 1½ hours had reached the level of 13°, in contrast to the previous case where the animal exposed to 14° did not reach 13.4° until late in the afternoon. The temperature continued to rise, but the temperature of 22° recorded late in the afternoon was still below the environmental temperature of 24°. Thus the marmot definitely approaches the cold-blooded animal, since it has a steady reaction in rectal temperature in approximate relation to the environmental temperature. In a number of instances the temperature of the environment, the respiration chamber or room, was inadvertently altered in an uncontrollable way, and invariably the rectal temperature was found to follow up and down the room temperature changes.

Although it is seen in the two cases referred to that the temperature rose from approximately 6.5° in one case to 13°, and in another case to 22°, in the course of 8 or 9 hours, in neither instance had the body temperature reached the environmental temperature at the time the measurements were discontinued. In a number of instances the marmots were taken back to the cold environment after this study. At times the animals woke up and remained awake, and in other instances returned to their hibernating state. The animal that showed a rectal temperature of 22° with an environment of 24° at 5.20 p.m., by the next morning at 9.00 a.m. had returned to the low level of 8.1°, with the garage temperature at about 5° C.

That the rectal temperature follows a decreasing room temperature is also shown with animal No. 26 on February 16. This animal was hibernating in a glass chamber in the refrigerator where the environmental temperature could be controlled at will. For some time it had been at a chamber temperature of 10° C., during which its rectal temperature was 11° C. When the chamber temperature was lowered, the rectal temperature slowly followed

it, but again it was always definitely behind the chamber temperature, as shown by the tabular presentation below.

Time	Chamber temperature °C.	Rectal temperature °C.
11.07	10.8	11.2
11.27	10.2	11.4
11.52	9.0	11.4
p.m.		
12.04	8.2	...
12.09	...	10.8
12.37	7.1	10.7
12.52	6.6	10.4
1.27	5.7	9.8
2.14	5.0	9.1
2.41	4.7	8.5
3.12	4.7	8.0
4.01	4.1	7.6
4.19	4.0	7.3
4.39	4.1	7.0
5.12	3.9	6.7

The rectal temperature usually follows the fluctuations of the environmental temperature, but very sluggishly. When the environmental temperature is rising, the rectal temperature is often temporarily below it. But when the environmental temperature is falling, this may be several degrees below the rectal temperature, due again to the sluggishness in reaction of the body temperature.

One of our earliest precautions was to insure that we were getting the true rectal temperature, *i.e.*, a temperature unaffected by the environment. For this study a thermo-junction was inserted, the temperature read, then the junction withdrawn gradually, and readings taken at different depths of insertion. Thus, on January 21, 1933 (environmental temperature 7°) with animal E, the thermometer was inserted to 15 cm. The temperature at this time was 24.9° C. On withdrawing the thermometer to 10 cm. the temperature was essentially the same, 25.0°, at 5 cm. it was 24.6°, and practically at the sphincter muscle, 22.6° C. The thermometer was again inserted at 9.03 a.m., read at 15 cm. as 25.2°, or 0.3° higher than at 8.57 a.m., an increase caused doubtless by the stimulation. At 10 cm. the temperature was 25.2°, at 5 cm., 24.9°, and at the exit, 23.4° C. It is thus clear that if the thermometer is inserted at least 5 cm., one can be certain that the true temperature of the hibernating marmot is being obtained. All the measurements of rectal temperature during hibernation, unless otherwise stated, met this requirement.

MINIMUM RECTAL TEMPERATURES

One of the most important factors in studying the rectal temperature was to note to what level the temperature of these animals can be lowered and the animal still survive. It has been the belief that these animals are stimulated by a temperature of about 10°, causing them to awaken, this being considered a measure of self-preservation. Second, it is believed that the animal succumbs at low temperatures, does not revive, and dies. It became important, therefore, to search our data with these points in mind.

Although most of our animals were maintained at an environmental temperature between 7° and 12° C., several animals were exposed to garage temperatures so low that their rectal temperatures descended to 5° or below. (See table 33.) In addition, one animal, No. 26, in the refrigerator was also in a similar environment. All but two of these animals noted in table 33 had temperatures at sometime of 4°, or lower, and two of the animals had the inconceivably low temperature of under 3° C. Animal D had a low tem-

TABLE 33—*Rectal temperatures below 6° C.—Hibernating marmots*
(Measured at intervals of 10 to 15 minutes)

Marmot and date	No. of observations	Temperature range	Marmot and date	No. of observations	Temperature range
1933		°C.	1933		°C.
D ¹			F ³		
Feb. 7	16	3.3-5.1	Feb. 11	14	2.8-4.1
" 8	1	4.8	" 13	1	4.1
" 20	10	5.0-6.0	" 14	15	3.7-5.1
E ²			" 15	15	4.6-5.8
Jan. 30	3	5.1-5.8	" 16	13	3.8-4.7
Feb. 7	2	4.4-4.7	" 20	1	5.4-5.9
" 10	13	2.5-4.7	" 23	6	5.2-5.9
" 13	1	6.0	March 1	12	4.7-6.0
" 14	7	5.2-6.0	" 2	13	5.2-5.7
" 15	5	4.9-6.0	" 3	14	4.3-5.6
" 16	12	4.4-5.3	" 4	1	4.5
" 17	17	3.8-5.4	" 8	1	5.9
" 18	1	5.1	G ⁴		
" 20	3	5.2-5.9	Jan. 16	1	5.5
March 2	1	5.2	Feb. 6	24	3.4-6.1
" 3	2	5.0-5.5	" 14	8	4.8-6.0
" 6	18	3.6-5.6	" 16	1	4.0
" 7	21	4.7-5.2	March 2	14	5.3-5.9
" 14	13	5.9-6.0	" 3	15	5.0-5.9
" 16	6	5.1-5.3	" 4	1	4.9
" 21	6	4.7-5.6	" 13	5	5.2-6.0
			" 14	19	5.0-6.0
			" 15	10	5.0-6.0
			" 16	12	4.4-5.8
		I			
		March 24 ⁵		1	5.8
		K			
		Feb. 8 ⁶		1	5.6
		" 26			
		Jan. 27 ⁷		18	4.0-6.2
		" 28		20	3.2-5.7

¹ Rectal temperature on Feb. 23 was 32° C.—died Feb. 25-27.

² Died March 21.

³ Rectal temperature on March 9 was 8.5° C.—died March 10.

⁴ Died March 20.

⁵ 1934; I died May 3, K on June 27.

⁶ Awake on Feb. 11—died March 1.

⁷ 1937.

perature on February 20, but on February 23 had recovered with a temperature of 32°, and did not die until about the 25th. Animal E, after the low temperature of 5.6° on March 21, woke up, attained a high temperature, was extremely active, and died that same day. With animal F the low temperature of 4.5° was found on March 4, yet the animal lived until March 10. Animal G showed temperatures under 5° on March 16, but lived until the

20th. With these marmots, E, F, and G, these low temperatures were obtained on a number of days, and the animals were awake between some of the observations recorded, which shows that the low cell temperatures were not necessarily the cause of death.

Animal 26 on the 27th and 28th of January showed minimum temperatures of 4.0° and 3.2° C., respectively, was awake on February 11, and did not die until March 1. Marmot E had a rectal temperature of 2.5° on February 10 and did not die until March 21. Animal F had a temperature of 2.8° on February 11, and did not die until March 10. It is clear that these animals may actually attain a rectal temperature as low as 5° or even 3° and still survive a considerable time after that.

No hint in our entire series of observations was found to suggest that the low temperatures, even as low as those recorded above, acted as a stimulus to waken the animals. Indeed, we see no reason why marmots should not be placed, in subsequent experiments, in a refrigerator at 0° without any danger of waking them.

TABLE 34—*Comparison of mouth and rectal temperatures of marmot No. 1, under nembutal*

Time	Temperature		
	Environmental °C.	Mouth °C.	Rectal °C.
a.m. 10.45	25.8	34.3	34.4
11.30	16.0	28.5	28.2
p.m. 12.30	15.2	22.4	22.4
1.30	13.8	20.7	20.6
2.20	13.0	19.3	19.3
3.20	12.8	18.0	18.0
4.08	11.8	17.1	17.0
5.05	12.1	16.7	16.7

RECTAL TEMPERATURE GRADIENT

As soon as the marmots that were drugged with nembutal became flaccid it was possible to insert thermo-junctions without stimulating them, and the thermo-junction would remain in place during the entire experiment. Because of the opportunity of handling the animals and trussing them up as desired, in a few cases a thermo-junction was inserted in the mouth as well as one in the rectum, which enabled a comparison of the temperatures at these two divergent points in the animal's body. The junction in the mouth was inserted 75 mm. and that in the rectum 150 mm.

The experiment with animal No. 1 on May 22, 1937, is representative and shows that over the entire range from 34.3° to 16.7° C. (see table 34) the temperatures of the mouth and the rectum were almost identical. In another instance in addition to the junctions inserted as before in the mouth and in the rectum, two other junctions were inserted in the rectum, one at 75 mm. and one at 25 mm. from the anus. The result of this study on marmot 2, on

May 27 and 28, is shown in figure 9. Here too it is seen that the body temperature measured at four positions in the body varied only one degree, and most of the time the difference was less than one-half a degree. On May 27 the body temperature was descending, and in the steady state on May 28 the agreement was still closer. As would be expected, the rectal junction nearest the anus was the coolest, since it was most influenced by the environmental temperature which in all cases was below the body temperature of the marmot.

Other experiments show maximum variations of one degree at these positions in the body. It can be concluded that the body temperature in

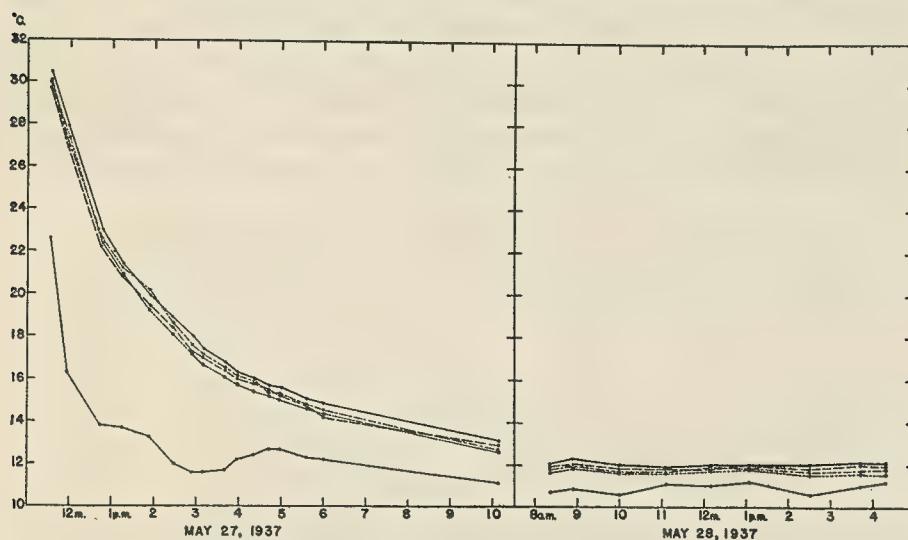


FIG 9—BODY TEMPERATURE OF MARMOT NO. 2, UNDER NEMBUTAL

The upper solid line represents the temperature in the mouth 75 mm. from the teeth; the broken line, the rectal temperature 150 mm. from the anus; the dash-dot line, the rectal temperature 75 mm. from the anus; the dotted line, the rectal temperature 25 mm. from the anus; and the lower solid line the environmental temperature. The relation of the temperatures at these four positions is shown on May 27, when the body temperature was profoundly lowered, and on May 28 when the temperature was at a constant level.

the static state is very uniform. This finding is not in agreement with the theory of some writers who think that the hind part of the body succumbs to cold first. At least as far as the body temperature is concerned there is no striking difference in the front and rear of the animal. It is highly probable that the body temperature of the normal hibernating animal in the steady state is uniform.

In connection with the gradient in the body, there is an extremely unusual finding with marmots 2 and 4 on May 25. These animals were drugged with nembutal on May 24. In addition to the intraperitoneal dose, each animal was given about one-third of a dose subcutaneously. When cooled, both ceased breathing. In order to assist in the restoration of the respiration,

the animals were immersed in water at 37° C., and the respiration was fairly readily restored by the use of oxygen and artificial respiration. The animals were then placed in a cage in a room which was maintained between 25° and 30° C. On examination the next morning (May 25), animal No. 4 appeared at first glance to be dead. However, the rate of five respirations in three minutes showed that it was still alive. Its rectal temperature, upon measurement, was found to be less than one degree lower than the room temperature, a difference which is not particularly significant. Table 35 shows the results obtained.

With animal No. 2 an even more striking differential was found. The thermometer inserted 150 mm. showed a temperature of 22.4°, four degrees below the room temperature. Withdrawing the thermometer half way resulted in a slight rise, and at 25 mm. from the anus a still higher temperature was noted. To prove this point conclusively the thermometer was then re-inserted to 75 mm. where a lower temperature was found, and a still further insertion to 150 mm. again showed essentially the temperature

TABLE 35—*Differences in rectal temperatures of marmots under nembutal*

Marmot	Environmental temperature	Depth from anus	Rectal temperature
2	°C. 26.6	mm. 150	°C. 22.4
		75	22.5
		25	22.8
		75	22.7
		150	22.5
4	26.7	mm. 150	°C. 25.8
		75	26.0
		25	25.9
		13	25.9
		150	26.0

first observed. In this case the temperature was appreciably below that of the room. A recording thermometer indicated that at no time during the animal's sojourn in the room had the room temperature been below 25° C. This rectal temperature of less than 23° shows that the animal had been receiving warmth from the room for some time. It will be noted that the temperature measured the deepest in the body was the lowest. This highly artificial series of events had produced a condition in these marmots which definitely resembles that of a cold-blooded animal, that is, the body temperature was appreciably below the environmental temperature. Our experience with nembutal has shown that the recovery from the influence of this drug, when the animal has been cooled under its influence, is extremely slow. Therefore a transitory condition still existed for these two animals, and this accounted for the body temperature apparently below the environmental temperature. It is regretted that no measures of the heat production were made under this condition.

RECTAL TEMPERATURES OF MARMOTS, COOLED, UNDER NEMBUTAL

Animal 24 was kept for a number of days under nembutal, and as will be shown later, the heat production was very constant, as was the rectal temperature which varied only from 11.1° to 12.9° C. The gradient between the environmental and the animal's cell temperature ranged between one and two degrees. Other marmots showed a similar picture of only one to three degrees. The minimum temperature which animals under nembutal attained was higher than that of animals normally hibernating. The lowest temperature observed under nembutal was 8.3° with animal 25. Several animals were cooled to 11.5° or 12.0° C. These temperatures are not necessarily the lowest at which these animals can be maintained under nembutal, but are due to the fact that the environmental temperature was maintained at higher levels than those frequently obtaining with normally hibernating animals. In one of the early attempts at drugging animals and subjecting them to a cold temperature, an influence of the size of the dose on the resulting body temperature is shown, for in the case of animal 19, on December 2, an injection of nembutal (in this case 0.6 c.c. or 39 mg. for a 1.72-kg. animal, instead of 1 c.c. or more), followed by subjection to an environmental temperature of 9° C., produced no appreciable variation from the normal rectal temperature. Unless the dose is large, no appreciable lowering of the temperature will be found.

There were occasions in the use of nembutal where it was impossible to lower the rectal temperature continuously, which may have been caused by the effect of the drug "wearing off". Thus, the dose is a factor in the rate and level to which the body temperature drops.

GENERAL CONCLUSIONS REGARDING THE RECTAL TEMPERATURE OF THE MARMOT DURING HIBERNATION

The marmot, when under nembutal and kept at a constant environmental temperature, has a rectal temperature 1° to 2° C. above that of the environment. The temperature in the body, at points from 25 to 150 mm. in the rectum and 75 mm. in the mouth, has been found to be very uniform, with a maximum variation of but 1° C. This striking uniformity is also noted during the transitory or cooling period.

In normal hibernation the rectal temperature of the marmot, at a constant environmental temperature, is always found to exceed the surrounding temperature by from 1° to 3° C. The body temperature may go as low as 3° C., after which it is possible for the animal to return to its warm-blooded state. It reacts slowly to the environmental temperature, but follows its general course.

ANALYSES OF THE URINES OF HIBERNATING MARMOTS**TOTAL NITROGEN IN URINE**

That the total metabolic activity of the marmot is lowered as it enters hibernation and that this lowered total metabolism is accompanied by a

profound decrease in rectal temperature, respiration rate, and heart rate, have already been demonstrated. The infrequency with which urine is passed and the small amounts passed make it highly probable that the protein metabolism during hibernation is also at a low level. Several marmots were kept in Abderhalden cages for a considerable length of time, during which time they were in various conditions, entering hibernation, hibernating, waking from hibernation, and remaining awake, with varying amounts of activity. The separation of the collections of urine according to these various stages was by no means perfect, but in most cases the collections could be made so as to distinguish approximately between these stages. The determinations of the total urinary nitrogen excreted by fasting and hibernating marmots, which is taken as the index of the protein metabolism, is recorded in tables 36 to 39, together with the body weights of the animals and the lengths of the collection periods. These amounts of total nitrogen per kilogram per 24 hours vary greatly, from as high as 300 mg. or more to as low as, in one instance (K from March 3 to April 7), 7 mg. In two other instances (E and F) low amounts of 4.6 and 7.6 mg. per kilogram per 24 hours were recorded. These results indicate the extremely low level to which the protein metabolism of the marmot may descend during hibernation. Those periods when the urine was collected during the deepest hibernation are indicated in tables 38 and 39 by the letter H.

The hibernating effect *per se* is obscured somewhat, as hibernation in all cases occurred only after considerable periods of fasting. The highest urinary nitrogen excretion usually appeared after a long period of hibernation and shortly before death. Absolutely the highest excretion during the fasting periods was that by marmot I on April 28 to May 3, namely, 659 mg. per kilogram per 24 hours. The animal died on May 3. Obviously the time element is always uncertain, as the precise moment of urination is unknown, but as most of the periods covered seven or more days, this factor does not play a great rôle. In the particular case of marmot I the period covered five days. The animal had been fasting a long time, its body weight had been greatly reduced, and the high nitrogen excretion is evidence of a premortal rise in the protein metabolism.

To attempt to establish a figure for the basal protein metabolism during true hibernation, uncontaminated by prolonged fasting, recourse may be had to the values obtained when these animals first hibernated. For E, the first two values obtained when it was in deep hibernation average 21 mg. per kilogram, covering a period of about two weeks, from December 24 to January 7. The lowest value with this animal, 4.6 mg., was found three weeks before the animal died. The first two values for F when in hibernation average 40 mg. The absolutely lowest value found with this animal of 7.6 mg. was obtained nearly three weeks later. Although every attempt was made to secure accurate collections, special stress would not be laid upon this low value were it not for the fact that similarly low values were found with at least two other marmots. With animal G the first three values

secured during hibernation average 35 mg. Marmot H did not hibernate. Marmot I from February 17 to April 14 excreted, on the average, 46 mg. of

TABLE 36—*Total nitrogen in urines of fasting marmots H, I, K, L, and M*

Marmot and date	Body weight (average)	Length of collection period	Nitrogen excretion per 24 hours		Remarks
			Total	Per kg.	
<i>1933-1934</i>					
	kg.	days	mg.	mg.	
H					
Jan. 20-27	2.46	7	228	92.7	Long fast began Jan. 16
" 27-Feb. 3	2.23	7	204	91.5	
Feb. 3-10	1.86	7	122	65.8	
" 10-17	1.81	7	223	123	
" 17-24	1.63	7	430	264	
" 24-Mar. 3	1.44	7	516	358	
Mar. 3-5	1.22	2	197	161	Died March 5
I					
Jan. 15-20	2.28	5	386	170	Long fast began Jan. 15
" 20-27	2.03	7	163	80.4	
" 27-Feb. 3	1.81	7	166	91.5	
Feb. 3-10	1.61	7	204	127	
" 10-17	1.49	7	113	75.7	
" 17-Mar. 10	1.33	21	52.2	39.2	Hibernating
Mar. 10-Apr. 14	1.12	35	58.4	52.1	"
Apr. 14-21	.99	7	247	250	
" 21-28	.91	7	451	496	
" 28-May 3	.79	5	521	659	Died May 3
K					
Jan. 13-20	1.55	7	115	74.3	Fasting began Jan. 13
" 20-27	1.45	7	55.2	38.1	
" 27-Feb. 3	1.36	7	52.8	38.8	
Feb. 3-Mar. 3	1.22	28	24.4	20.0	Hibernating
Mar. 3-Apr. 7	1.10	35	7.7	7.0	"
May 5-12	.84	7	43.2	51.4	
" 12-19	.78	7	28.8	36.9	
" 19-26	.72	7	79.2	110	
" 26-June 2	.67	7	134	201	
June 2-9	.62	7	106	170	
" 9-16	.56	7	86.4	154	
" 16-23	.52	7	113	217	
" 23-29	.48	6	144	300	Died June 29
<i>1934-1935</i>					
L					
Nov. 14-21	2.14	7	133	62.1	Arrived at Laboratory
" 21-30	1.86	9	181	97.3	Nov. 14. No food.
" 30-Dec. 7	1.80	7	162	89.9	
Dec. 7-Jan. 7	1.63	30	34.1	20.9	Hibernating
Jan. 7-Feb. 16	1.43	40	17.0	11.9	"
" 16-Mar. 23	1.36	35	14.4	10.6	"
Mar. 30-Apr. 6	1.32	14	11.9	9.0	"
M					
July 27-Aug. 9	1.27	13	64.6	50.8	Hibernating

nitrogen per kilogram during hibernation. This picture, however, is complicated by fasting, during which the body weight had decreased to half the initial weight. The amounts of nitrogen excreted by L during hibernation

were low, 20.9, 11.9, 10.6, and 9.0 mg. Thus it would appear that the marmot in deep hibernation may have a nitrogen excretion as low as 7 mg. per kilogram per 24 hours. This, however, is undoubtedly affected by prolonged fasting. At the first onset of hibernation, before the deepest inroads of fasting, the excretion will be more nearly 40 or 45 mg. Marmot B (table 37), which fasted but did not hibernate, during the period from July 5 to September 23 had a reasonably uniform nitrogen output per kilogram of about 45 mg. According to this finding, the level in prolonged fasting may approach the low level found in some phases of hibernation.

From the data obtained it is concluded that the marmot's nitrogen excretion during deep hibernation (7 mg.) is only about one-tenth that when the

TABLE 37—*Nitrogen in urine of fasting marmot B*

Date	Body weight (average)	Length of collection period		Nitrogen excretion per 24 hours	
		days	hr.	Total	Per kg.
1932	kg.			mg.	mg.
June 27 ¹	3.29	0	0		
June 27-June 28	3.18	1	6	1741	547.4
June 28-July 1	3.00	2	19	484	161.2
July 1-July 5	2.88	4		161	55.8
July 5-July 8	2.85	3		203	71.2
July 8-July 9	2.83	1	6	229	80.9
July 11-July 15	2.72	4		162	59.5
July 15-July 18	2.68	3		79	29.6
July 18-July 21	2.65	3		173	65.2
July 21-July 25	2.58	4	6	113	43.7
July 25-July 29	2.54	3	19	100	39.2
July 29-Aug. 1	2.49	3		126	50.5
Aug. 1-Aug. 5	2.43	4		111	45.8
Aug. 5-Aug. 8	2.40	3		72	30.0
Aug. 8-Aug. 13	2.37	5	3	118	49.8
Aug. 13-Aug. 19	2.33	5	21	86	37.1
Aug. 19-Aug. 24	2.27	5	5	80	35.1
Aug. 24-Aug. 31	2.20	5	23	81	37.0
Aug. 31-Sept. 7	2.14	7	1	52	24.4
Sept. 7-Sept. 14	2.07	7		64	30.9
Sept. 14-Sept. 23	2.06	8	19	69	33.4
Sept. 23-Sept. 30 ²	2.00	7		187	93.7

¹ Started long fast on June 27.

² Died October 1.

animal is awake but has been fasting for a long time (see page 84). Evidently hibernation, as such, has a tremendous influence. However, even this low output is probably contaminated by a certain percentage of protein metabolism occurring in the period when the marmot is entering hibernation, which may be as long as 12 hours. As the collection periods were usually 7 days in length, it is obvious that during one-fourteenth of this time the nitrogen excretion might have been influenced by the transitional stage of entering hibernation, including the effect of prolonged fasting. This would not show the effect of hibernation *per se*. If the urine could be collected in periods of deep hibernation uncontaminated by transitional stages, it is believed that the nitrogen excretion under such conditions would be somewhat

lower than 7 mg. The possibility of finding too low values due to the fact that periods did not start and end at the time of voiding is recognized.

Partition of urinary nitrogen

The earliest record regarding the urine of hibernating animals is that of Valentin,¹⁴ who determined only the percentage by weight of urea in the urine. The only systematic study of the urines of marmots during hibernation was made by Nagai,¹⁵ who used the method of Pfaundler¹⁶ for separating the nitrogen into fractions by means of phospho tungstic acid. He found a shift in the so-called "ammonia" fraction in that in hibernation it was almost half that during fasting, and that the "amino-acid" fraction was markedly increased during hibernation as compared with that found during fasting or when the animal was on food.

Our colleague, Dr. Thorne M. Carpenter, kindly assumed the responsibility for an illuminating study of the partition of the urinary nitrogen of marmots D, E, F, and G. We are extremely grateful to him for his report,^{17a} which follows.

The amino-acid nitrogen, ammonia nitrogen, urea nitrogen, and preformed and total creatinine were determined in the marmots' urines collected during fasting and hibernation. The methods used were those of Folin and his associates.^{17b}

Ammonia nitrogen. In tables 38 and 39 the ammonia nitrogens are given as percentages of the total nitrogen excreted. The percentage of total nitrogen as ammonia in urine depends upon the adequate preservation of the urine at the time of collection, upon the level of nitrogen excretion, that is, as to whether the animal is on a high nitrogen level or is approaching a condition in which the nitrogenous metabolism is nearly that represented by the endogenous level, and upon the elimination of organic acids, such as beta-oxybutyric acid. In the urines of D the nitrogen eliminated as ammonia ranged from 12.2 per cent during the two days of November 15 to November 17 to 4.1 per cent on November 21 to 23 and during the period from February 6 to 15. With the possible exception of the highest value and that obtained during December 5 to 7 the percentages do not indicate any spoilage of samples. With E the range during the fasting period as well as during hibernation is from 14.0 to 4.3 per cent. The percentages of nitrogen as ammonia nitrogen with E are in general higher than those with D, and a number of them indicate possible spoilage of urine, that is, those above 9 per cent. With F during the fasting and hibernating period the percentages range from 14.5 in the period from January 21 to 28 to 4.4 in the last period

¹⁴ Valentin, G., Moleschott's Untersuchungen, 1857, **2**, p. 1. Cited from Ferdmann, D., and O. Feinschmidt, Ergebnisse der Biologie, 1932, **8**, p. 28.

¹⁵ Nagai, H., Zeitschr. f. allg. Physiol., 1909, **9**, p. 306.

¹⁶ Pfaundler, M., Zeitschr. f. physiol. Chem., 1900, **30**, p. 75.

^{17a} An abbreviated report has recently appeared. (See Carpenter, T. M., Journ. Biol. Chem., 1938, **122**, p. 343.)

^{17b} Folin, O., *Laboratory manual of biological chemistry*, New York, 5th ed., 1934.

of the fast, March 6 to 7. The highest value is probably erroneous due to decomposition of the urine. With G the values are much more uniform than with the other animals, ranging from 4.5 to 6.4 per cent of the total

TABLE 38—*Partition of nitrogen excreted in urines of marmots D and E*

Marmot and collection period ¹	Days fasting at start of period ²	Body weight (average)	Volume of urine collected	Amounts per 24 hours			Proportion of total nitrogen as				Creatinine coefficient ³
				Total nitrogen	Total nitrogen per kg.	Creatine as creatinine	Ammonia	Amino acid	Urea	Preformed creatinine	
kg.	c.c.	mg.	mg.	mg.	p. ct.	p. ct.	p. ct.	p. ct.	p. ct.	mg.	
<i>1932-1933</i>											
<i>Marmot D</i>											
Nov. 15-Nov. 17		1.70	352	614	361	53.9	1.47	12.2	1.1	79.0	1.6
" 17- " 19		1.61	130	504	313	48.6	33.2	4.2	6.86	31.8	1.2
" 19- " 21		1.52	172	373	242	47.4	.69	4.2	.585	1.24	0.0
" 21- " 23		1.48	100	316	214	47.6	26.5	4.1	.984	6.28	1.6
" 23- " 28		1.40	100	260	186	91.3	87.3	4.7	.582	2.26	2.5
" 28-Dec. 3		1.24	267	264	213	84.5	141	5.1	.685	9.24	4.0
Dec. 3- " 5		1.25	100	266	213	32.3	40.2	7.7	.982	0.23	2.8
" 5- " 7		1.28	221	866	677	70.7	34.5	11.4	.881	9.15	.7
" 7- " 9		1.28	166	637	498	39.3	14.1	7.1	.687	7.11	.4
" 9- " 12		1.25	270	836	669	71.1	19.0	6.2	.984	8.11	.3
" 12- " 14		1.30	287	684	526	39.7	36.8	5.5	.883	6.11	1.0
" 14- " 17		1.23	102	375	307	43.9	32.8	4.9	.585	1.15	1.1
" 17- " 20		1.13	123	336	297	45.6	88.1	5.2	.680	0.17	3.3
" 20- " 31	(H)	1.13	105	32.9	29.1	18.8	2.46	5.2	.780	9.19	.3
" 31-Jan. 7		1.05	101	62.1	59.1	22.2	2.59	4.6	.676	0.19	.2
Jan. 7- " 14		1.02	110	37.1	36.4	14.4	2.94	4.8	1.089	0.21	.4
" 14- " 16		1.02	98	111	109	10.3	3.71	4.8	1.085	6.17	.6
" 16- " 21	(H)	1.02	95	49.8	48.8	12.3	3.44	4.8	1.081	3.18	.5
" 21- " 30		.99	95	60.3	60.9	23.2	6.29	4.5	1.074	5.16	.4
" 30-Feb. 4	(H)	.96	99	49.0	51.0	11.7	1.66	4.8	1.088	1.8	.3
Feb. 4- " 6		.94	99	201	214	14.1	1.76	4.7	.680	8.13	.2
" 6- " 15		.91	97	85.2	93.6	19.3	8.22	4.1	.876	6.9	.4
" 15- " 24 ⁵	(H)	.91	109	29.2	32.1	12.5	7.06	4.3	1.084	3.18	1.0
<i>Marmot E</i>											
Dec. 8-Dec. 10		2.58	198	1135	440						
" 10- " 12		2.58	466	1707	662						
" 12- " 14		2.64	329	1181	448						
" 14- " 17		2.48	302	656	264	83.9	24.9	10.2	1.1	80.6	1.6
" 17- " 20		2.37	211	281	355	81.2	24.4	6.1	.881	3.36	1.1
" 20- " 24		2.29	106	121	53.0	73.1	4.27	9.3	.775	2.56	.3
" 24- " 31	(H)	2.17	132	56.5	26.1	62.9	6.98	11.7	.573	1.59	.7
" 31-Jan. 7	(H)	2.07	100	32.2	15.6	42.6	7.70	5.0	.859	7.0	1.3
Jan. 7- " 14		2.01	381	99.7	49.6	115	27.0	14.0	.971	3.6	1.4
" 14- " 16	(H)	1.94	99	82.7	42.6	22.1	5.77	8.0	.678	5.0	1.3
" 16- " 21	(H)	1.93	100	69.4	36.0	6.6	6.70	5.6	.780	0	.7
" 21- " 28	(H)	1.90	215	58.2	30.6	63.7	18.2	11.9	.719	5.8	1.7
" 28-Feb. 8	(H)	1.83	94	30.2	16.5	39.2	8.30	5.7	.7	4.3	.9
Feb. 8- " 21	(H)	1.80	97	19.5	10.8	30.3	14.4	4.3	.977	6.44	2.1
" 21-Mar. 1 ⁶	(H)	1.78	98	8.1	4.6	⁷		77.5			1.3

¹ Each period began and ended at 9 a.m.

² The letter H in parentheses against the days fasting indicates those periods in which the marmot was in deep hibernation.

³ Preformed creatinine per 24 hours divided by body weight.

⁴ Prolonged fasting began at 9 a.m., Dec. 12.

⁵ Marmot D died on Feb. 27.

⁶ Marmot E died on March 22.

⁷ Trace too small to determine.

nitrogen. In general, the amount of nitrogen as ammonia in the urines of fasting and hibernating marmots is in the neighborhood of 5 per cent and the values are not affected either by the duration of the fast or the condition of

TABLE 39—*Partition of nitrogen excreted in urines of marmots F and G*

Marmot and collection period ¹	Days fasting at start of period ²	Body weight (average)	Volume of urine collected	Amounts per 24 hours			Proportion of total nitrogen as									
				Total nitrogen	Total nitrogen per kg.	Preformed creatinine	Creatine as creatinine	Ammonia	Amino acid	Urea	Preformed creatinine	Creatine	Creatinine coefficient ³			
				kg.	c.c.	mg.	mg.	p. ct.	p. ct.	p. ct.	p. ct.	p. ct.	mg.			
<i>1932-1933</i>																
<i>Marmot F</i>																
Dec. 12-Dec. 14	40	1.81	308	904	500	83.5	5.41	7.8	0.5	74.6	2.0	0.1	17			
Dec. 14-Dec. 17	2	1.67	144	529	317	29.5	17.8	9.2	.7	83.1	2.7	1.6	6.6			
Dec. 17-Dec. 20	5	1.50	107	134	89.4	29.5	8.83	4.9	.6	81.4	2.5	.3	13			
Dec. 20-Dec. 24	8	1.44	111	272	189	74.0	4.60	5.2	.7	76.0	2.2	.5	2.3			
Dec. 24-Dec. 31	(H) 12	1.37	102	53.3	38.9	22.4	4.45	4.7	.6	74.5	1.9	.4	2.0			
Dec. 31-Jan. 7	(H) 19	1.37	98	55.2	40.3	19.6	4.45	4.7	.6	74.4	1.9	.4	2.0			
Jan. 7-Jan. 14	26	1.29	106	112	86.5	71.9	6.66	5.2	1.1	88.4	3.4	.3	8.0			
Jan. 14-Jan. 21	33	1.22	100	85.0	69.6	47.6	6.15	4.8	.8	78.6	3.0	.4	5.6			
Jan. 21-Jan. 28	(H) 40	1.19	99	9.0	7.6	7.6	2.58	14.5		64.3	4.5	1.5	.9			
Jan. 28-Feb. 4	(H) 47	1.15	99	28.2	24.5	16.9	2.03	5.3		81.9	3.1	.4	2.1			
Feb. 4-Feb. 7	54	1.16	98	96.9	83.5	17.0	2.66	5.4	.9	75.2	2.2	.3	4.9			
Feb. 7-Feb. 17 ⁵	(H) 57	1.15	125	33.6	29.2	12.8	3.85	4.6	1.3	81.0	1.4	.4	1.1			
Feb. 17-Feb. 24 ⁶	(H) 67	1.12	97	39.4	35.2	11.4	2.20	5.3	1.3	71.8	1.6	.3	1.5			
Mar. 1-Mar. 6 ⁷	79	1.05	156	324	308	71.9	24.4	8.2	.6	80.2	1.6	.6	13			
Mar. 6-Mar. 7 ⁸	84	1.02	98	472	463	22.8	10.2	4.4	.6	83.4	1.9	.9	24			
<i>Marmot G</i>																
Dec. 12-Dec. 14	40	1.11	189	578	521											
Dec. 14-Dec. 17	2	1.08	101	233	215	43.2	11.7	5.1	0.8	81.8	2.3	0.6	13			
Dec. 17-Dec. 20	5	1.00	103	115	115	23.4	16.5	5.8	1.1	75.2	2.5	1.8	7.8			
Dec. 20-Dec. 24	8	.88	114	117	132	29.0	14.8	5.4	1.2	79.9	2.3	1.2	8.2			
Dec. 24-Dec. 31	12	.78	114	101	129	43.0	13.7	6.4	.9	82.6	2.3	.7	7.9			
Dec. 31-Jan. 7	19	.68	100	36.4	53.5	20.0	7.03	4.5	1.0	80.9	2.9	1.0	4.2			
Jan. 7-Jan. 14	26	.66	101	55.2	83.6	26.9	11.6	4.9	.7	86.3	2.6	1.1	5.8			
Jan. 14-Jan. 21	(H) 33	.63	100	23.8	37.7	11.4	2.15	5.8		81.7	2.5	.5	2.6			
Jan. 21-Jan. 28	(H) 40	.61	103	24.9	40.9	7.23	3.90	6.0		85.2	1.5	.8	1.7			
Jan. 28-Feb. 4	(H) 47	.60	97	16.1	26.8	9.27	.78	5.1		80.4	3.1	.3	2.2			
Feb. 4-Feb. 7	54	.60	93	44.3	73.8	9.32	3.08	5.0		73.7	2.6	.9	5.2			
Feb. 7-Feb. 17	(H) 57	.59	95	12.4	21.0	7.83	2.36	6.2		74.6	2.3	.7	1.3			
Feb. 17-Feb. 28	67	.57	92	15.6	27.4	6.24	3.34	5.4		72.3	1.4	.7	1.0			
Feb. 28-Mar. 7 ⁹	(H) 78	.54	92	20.5	37.9	5.54	2.85	4.9		76.3	1.4	.7	1.5			

¹ Each period began and ended at 9 a.m., except where otherwise noted.

² The letter H (in parentheses) against the days fasting indicates those periods in which the marmot was in deep hibernation.

³ Preformed creatinine per 24 hours divided by body weight.

⁴ Prolonged fasting began at 9 a.m., Dec. 12.

⁵ Period began at 9 a.m., Feb. 7, and ended at 4.30 p.m., Feb. 17.

⁶ Period began at 4.30 p.m., Feb. 17, and ended at 12.30 p.m., Feb. 24.

⁷ Period began at 9 a.m., Mar. 1, and ended at 10.45 a.m., Mar. 6.

⁸ Period began at 10.45 a.m., Mar. 6, and ended at 9 a.m., Mar. 7; marmot F died March 10.

⁹ Marmot G died on March 20.

the animal as to whether it was awake or hibernating. Also they are not appreciably affected by the general level of nitrogen metabolism, for with D in the period between November 15 and December 12 the nitrogen excre-

tion per kilogram per 24 hours was far higher than during the long fast when it fell to an extraordinarily low level, and yet the percentage of ammonia nitrogen was not significantly changed. In the urine of a fasting man, studied at this Laboratory,¹⁸ the proportion of total nitrogen as ammonia was far higher. At the beginning of the fast it was somewhat like that noted with these marmots, namely, from 4 to 5 per cent. With increasing duration of the fast, however, it rapidly approached a much higher level, and between the 16th and the 20th days of this man's fast it constituted 20 to 21 per cent of the total nitrogen. Simultaneously there had also occurred from the 3rd to the 20th day a fall in nitrogen excretion from 11.3 to 7.7 gm. Thus part of the rise in percentage is due to a falling nitrogen level. In studies with fasting steers¹⁹ the percentage of nitrogen as ammonia was slightly lower, on the whole, than with the marmots, particularly with the adult steers with which in a fast of ten days the values ranged from 1.1 to 7.7 per cent of total nitrogen. With an elephant on food studied by the Nutrition Laboratory²⁰ the ammonia nitrogen ranged from 1.0 to 2.9 per cent of the total nitrogen.

Amino-acid nitrogen. The amino-acid nitrogen of D constituted from 0.5 to 1.1 per cent of the total nitrogen eliminated in the urine, that of E from 0.5 to 1.1 per cent, that of F from 0.5 to 1.3 per cent, and that of G (for which the series is not so complete) slightly higher, namely, from 0.7 to 1.2 per cent. As with the ammonia nitrogen, the percentage of nitrogen as amino acid is not affected by the condition of nourishment (whether the marmot has had food or is fasting), by the duration of the fast, or by hibernation. There is no consistent change in the percentage due to any one of these factors. In the fast with the two adult steers, the amino acid constituted from 0.5 to 6.0 per cent of the total nitrogen. After the first three or four days of fasting the values approached a fairly uniform level of 0.6 to 0.7 per cent of the total nitrogen.

Urea nitrogen. When animals are on a high protein level, urea nitrogen may constitute over 80 per cent of the nitrogenous elimination in the urine. When, however, they are on a high carbohydrate-fat diet, which is adequate for energy requirements but represents a low protein intake, the percentage of nitrogen as urea in the urine falls to low values because a condition is approached where the nitrogenous metabolism is almost entirely endogenous. In the latter case the elimination of ammonia, uric acid, and creatinine bodies constitutes a greater percentage of the urine. With D the nitrogen as urea varied from 74.5 to 89 per cent of the total urinary nitrogen. With E the values range from 67.8 to 85.9 per cent and are, on the whole, lower than with D. With F they range from 64.3 to 88.4, and with G from 72.3 to 86.3 per cent. With D there is no indication that extraordinarily large changes in the nitrogen excretion, either the total excretion per day or the excretion per kilogram, resulted in any alteration in the percentage of nitro-

¹⁸ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 203, 1915, p. 256.

¹⁹ Benedict, F. G., and E. G. Ritzman, Carnegie Inst. Wash. Pub. No. 377, 1927, p. 117.

²⁰ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 474, 1936, p. 219.

gen as urea, and there is no indication that the condition of nutrition (whether the marmot had food or was without food and whether hibernating or not) affected the proportion of nitrogen as urea. With E there is no definite change in the percentages. Similarly with F, although there are some variations in the values from period to period, there is no directional change in the percentage of nitrogen as urea. With G the values from the 54th day to the end of the fast are slightly lower than the values in the period preceding. During the 31-day fast by the human subject studied at the Nutrition Laboratory, the urea nitrogen constituted from 80 per cent of the total urinary nitrogen at the beginning of the fast to 66.7 per cent on the 19th day, and with the fasting adult steers in 10 days from 26.4 to 75.5 per cent.

Preformed creatinine. The determination of creatinine elimination in the urine is of importance because of its indication as to the condition of nutrition of the animal, namely, whether it is excessively fat or undernourished. With excessively fat individuals or animals the amounts eliminated per kilogram per 24 hours are apt to be lower than the general average, whereas with undernourished individuals who, however, are still in good muscular condition the amounts are apt to range higher than the average. The determination of creatinine elimination is also of importance because of the growing belief that there is a relationship between the basal metabolism and the creatinine elimination of the individual. It also forms a useful criterion as to whether the urine collection has been adequate and complete. When animals are on a high protein diet the percentage of nitrogen as creatinine is relatively low, but when the nitrogen elimination approaches the endogenous level the percentage of nitrogen as creatinine increases markedly because the total creatinine per day is not changed by the protein level. With D the proportion of nitrogen as preformed creatinine varied from 0.9 to 2.8 per cent. In general, in the period from November 19 to December 5 the percentages averaged higher than during the remainder of the time. Other than this period there is no indication of change in the percentage of nitrogen as creatinine, in spite of the extraordinarily wide differences in the elimination of total nitrogen per day and per kilogram per 24 hours, the latter varying from 677 mg. on December 5 to 7 to 29 gm. from December 20 to 31. With E the proportion of total nitrogen as preformed creatinine is, on the whole, much higher than with D. With the exception of two instances it constitutes from 3.6 to 7.0 per cent of the total nitrogen. Marmot F shows proportions of preformed creatinine in total nitrogen from 1.4 to 4.5 per cent, and G from 1.4 to 3.1 per cent.

Creatine. In all the periods with all four marmots there was an elimination of creatine, and it constituted a relatively large percentage of the total creatinine elimination. In some cases the creatine elimination exceeded the creatinine elimination. With D there was a tendency for lower amounts after the eighth day of the fast. With the other marmots there was no consistent change.

Creatinine coefficient. The creatinine coefficient is of importance because

it is considered to be an index of the active protoplasmic tissue and also an indication of reserve material, that is to say, whether the animal is fat or lean. The fatter the animal the lower will be the creatinine coefficient. It is usually calculated by dividing the total creatinine eliminated per 24 hours by the body weight and is expressed as milligrams per kilogram of body weight per 24 hours. With D before the long fast began on December 12 the coefficients ranged, for the most part, between 13 and 19 mg. per kilogram of body weight. For an unknown reason there is an exceptionally high value of 28 for the period from December 5 to December 7. This presumably is coupled with the high nitrogen elimination for that period, as the values of all the other nitrogenous constituents of the urine are high. There is then no appreciable change in the coefficient until the period of December 20 to 31, which starts on the eighth day of fasting. Another use of the creatinine coefficient is that it serves as an index as to the complete collection of the urine and the proper spacing of the time of collection. It would seem from the coefficients up to December 20, with the exception of the high value of 28, that the urines had been adequately collected and that the separation, for the most part, corresponded to nearly the exact times of voiding. The marmot first hibernated in the period from December 20 to 31, when there is an extraordinary drop in the coefficient from 13 in the previous period to 1.5. During the entire remainder of the fast and hibernation all the coefficients are low, the highest being 7.5 in the period from February 4 to 6. There is no evidence that the results in the periods of hibernation and fasting vary from those in the periods of fasting alone.

With E at the beginning of the fast in the two periods from December 14 to 20 the coefficients are 11 mg., or slightly lower than the general average found with D. In the next two periods, December 20 to December 31, the coefficient decreases materially and in the following period from December 31 to January 7 (hibernation), it reaches the low level of 2.9 mg. In the next period, January 7 to 14, it returns to 8.2 mg. Judging from this factor alone one would infer that E was not hibernating during this period but that it had temporarily returned to a condition somewhat like that in the early part of the fast. This is substantiated by the fact that the nitrogen excretion per kilogram per 24 hours in the period from December 20 to 24 was 53.0 mg. and in the period from January 7 to 14, 49.6 mg. Low values for the creatinine coefficient are again reached in the latter part of the fast and during the hibernating period.

With F, the first value obtained is 17 mg. in the period from December 14 to 17, *i.e.*, on the second to the fifth day of fasting. In the next period there is a sharp drop to 6.6 and then a rise again to 13 mg. in the period from December 20 to 24. The maximum values, therefore, for this animal are somewhat similar to those for D. The effect of hibernation is immediately shown in the period from December 24 to 31, when the coefficient drops to 2.3 mg. From there on in all the periods of hibernation there are low values. In the period from February 4 to 7 the value is slightly higher than in the

hibernating periods, but it is apparent that marmot F had not returned to a condition of nitrogen metabolism anything like that occurring at the beginning of the fast. High values are again obtained in the last two periods from March 1 to 7. These accompany also extraordinarily high values for nitrogen excretion per kilogram per 24 hours. As marmot F died three days after the end of the last period, it would seem as though the nitrogen metabolism during the last seven days of the collection of urine was of the character of a premortal rise. In this condition there is apparently a return, so far as the creatinine coefficient is concerned, to a level like that occurring at the beginning of the fast. In addition, the nitrogen distribution in these two periods is not materially different, percentagewise, from that either at the beginning of the fast or during the periods of hibernation. From these two periods alone it would seem that the premortal rise in nitrogen metabolism does not result in a marked change in the distribution of the nitrogenous constituents.

With G the first value in the period beginning December 14, that is, on the second day of the fast, is 13 mg., which is of about the same order of magnitude as with D, E, and F. In all the other periods, however, marmot G has distinctly lower creatinine coefficients, varying between 8.2 and 1.0 in the entire 85 days of the fast. The low nitrogen excretion per kilogram and the low creatinine coefficients indicate that the animal must have been in deep hibernation for most of this time.

With the fasting steers²¹ the creatinine coefficient varied between 17 and 29 mg., and there was a tendency for the value to increase during the fasting period. With the fasting man studied at the Nutrition Laboratory, the creatinine coefficient varied from 21.5 on the first day to 25.3 on the fourth day and gradually decreased until the minimum was found on the 31st day of the fast, namely, 18.1 mg. The creatinine coefficient of sheep studied by Palladin^{22a} varied during fasting from 15.5 to 20.0. That of the elephant Jap (weight 3672 kg.) studied in this Laboratory was 4.5 mg. Marmots in the condition of full feed or at the beginning of a fast have creatinine coefficients ranging from 11 to 19 mg., or slightly lower than the coefficients of either man or steers, similar to those of sheep, and much higher than that of the elephant.

General conclusions. Comparison of the nitrogen distribution of the urines of marmots at the beginning of fasting and in the fasting and hibernating conditions shows that, in general, there is no particular shift in the distribution of nitrogenous constituents of urine so far as the percentage of total nitrogen eliminated is concerned. Neither the duration of the fast nor the condition of hibernation has any apparent effect on the percentages of nitrogen in the form of ammonia, amino acids, urea, and preformed creatinine. Both fasting and hibernation simply resulted in a general lowering of the pro-

²¹ Benedict, F. G., and E. G. Ritzman, Carnegie Inst. Wash. Pub. No. 377, 1927, p. 122; Carpenter, T. M., Am. Journ. Physiol., 1927, 81, p. 538.

^{22a} Palladin, A., Arch. f. d. ges. Physiol., 1924, 203, p. 96; *ibid.*, 1924, 204, p. 155.

tein metabolism without altering the general character of the protein metabolized. This indicates that there was a decrease in the total protein metabolism accompanying a decrease in the total energy metabolism. The kind of protein metabolized, therefore, during hibernation and fasting of marmots does not differ from that metabolized at the beginning of the fasting period.

GASEOUS METABOLISM OF THE HIBERNATING MARMOT

Because of the low levels in the rectal temperature, respiration rate, and heart rate of the marmot when in hibernation and because of the lowering of the gaseous metabolism when the animal is entering into hibernation, special interest falls upon the absolute level to which the metabolism descends when these animals are in profound torpor. This interest is twofold, the first concern being to note whether there is any relationship between the cell temperature and the metabolism. Perhaps the greatest interest, however, is in the question as to how closely the metabolic level of the hibernating marmot can approach that of cold-blooded animals of similar weight and of essentially the same cell temperature, thus furnishing anchorage (to retain the simile) at the cold-blooded end of the chasm between the cold-blooded and warm-blooded animals. Since the marmot is the keystone in a possible arch of the bridge, it is necessary to discuss it here, although this is more or less anticipatory of the more elaborate discussion in a forthcoming publication on the interspecific comparison of metabolism.^{22b} The respiratory exchange of the marmot, expressed in terms of the 24-hour heat production per $10w^{\frac{1}{2}}$, has already been shown to be very labile, both when the marmot is fully awake and when in the transition stage of entering hibernation. The non-hibernating marmot reacts to any deviation from the critical temperature and shows a chemical regulation of its heat production at the low environmental temperature at which these animals are usually maintained in hibernation. As the marmot enters hibernation, there is a lessening of the chemical regulation, causing a fall in metabolism which takes place even when the animal is in an environment as cold as 3° or 4° C. In the experiments made during this transition stage (cited on page 107) the heat production fell from an initial level of 1000 or 1500 calories to 500 calories or even as low as 200 calories per $10w^{\frac{1}{2}}$ during a period of observation of about $6\frac{1}{2}$ hours. These particular marmots were not studied continuously, however, until they reached a uniformly low metabolic level, which was a defect in the observations. In fact, none of the studies during this transition stage were continued until the rectal temperature of the animal was significantly low, that is, approximating that found during deep hibernation. Only when all the general criteria used in establishing deep hibernation prevail can the minimum heat production be expected. These criteria are (1) a slow respiration rate, in the neighborhood of 1 or less per minute, (2) a heart rate of about 5 or 6 per minute, unfortunately measured only with difficulty, and (3) a rectal temperature of 12° or below.

^{22b} Benedict, F. G., Carnegie Inst. Wash. Pub. No. 503, 1938.

Many of the marmots did not enter into deep hibernation until they had been fasting 30 days or more, but a study of the energy production during prolonged fasting shows that deprivation of food for 30 days has no profound effect on the heat production per $10w^3$ of the non-hibernating marmot. To separate completely the effects of hibernation and prolonged fasting is seemingly impracticable. The intermediary stage of entering hibernation, when the respiration rate, rectal temperature, and heat production are variable, has been discussed already (page 107), but the chief interest at present is in the metabolism during deepest hibernation. Further discussion of transitory stages will be made later (page 189) when the results of the large number of experiments with marmots awakening from hibernation are presented.

As frequently emphasized, the maintenance of a marmot in the true hibernating condition for a long period in the laboratory is difficult. On the other hand, it is feasible to disturb or stimulate a hibernating marmot designedly and measure its metabolism during the stage of awakening from hibernation. Obviously those studies on marmots in deep hibernation, in which all the physiological factors, heart rate, respiration rate, rectal temperature and gaseous metabolism were simultaneously measured, have the greatest value. Unfortunately there were only a few instances where it was possible to do this. Although some respiration rates were observed and the rectal temperature was occasionally recorded at the end of the experiment, the heart rate could be measured only late in the investigation.

The development of techniques for determining precisely the extremely small amounts of carbon dioxide produced and oxygen consumed by these animals in deep hibernation taxed our ingenuity and the laboratory resources for years. It has been shown that both when the marmot is entering hibernation and is in hibernation the combustion process is one of fat, resulting in a respiratory quotient of about 0.70,²³ and deviations from this quotient are ascribable in large part to errors in the determination of the carbon-dioxide production. As the calculations of heat production are based almost exclusively upon the oxygen measurements, however, one can rely upon the accuracy of the heat calculations in discussing the energy relations of these animals.

As it has already been pointed out in the discussion of the body weight changes in hibernation that it is possible for the fur of the marmot to take on or give off water, and as the quantity of heat which is being produced is so small, it is evident that attempts to measure the amount of water vaporized during hibernation (to establish the percentage of heat production that is lost through this path) will have no significance.

The data obtained on marmots in deep hibernation are reported in table 40. Although the average body weight of all the marmots when normally

²³ Throughout this manuscript this quotient or 0.71 is used as an indication of the fat quotient. It is obvious that rarely, if ever, is there an exclusive fat combustion in hibernation or during fasting. There is invariably a certain proportion of protein oxidized, which may represent not far from 10 to 15 per cent of the total energy production.

feeding was found to be 3.1 kg., the weights at the time of these hibernation experiments were all under 2 kg. Two factors contribute to this. In the first place, the marmots frequently did not hibernate until after a relatively long fast, and the rapid loss in weight during the early stages of prolonged fast would account in part for the small body weights. Secondly, although

TABLE 40.—*Energy metabolism of marmots during deep hibernation*

Marmot and date	Body weight	Rectal temperature ¹	Minimum heat production per $10w^{2/3}$ per 24 hours	Marmot and date	Body weight	Rectal temperature ¹	Minimum heat production per $10w^{2/3}$ per 24 hours
	kg.	°C.	cal.		kg.	°C.	cal.
A—1932				F—1933			
January 23	1.25	(9.6)	36	December 27 ²	1.39	7.7	80
January 25		(9.4)	60	January 5		11.3	26
January 26		(9.9)	80	January 18	1.19	9.4	27
				January 19		(8.3)	32
B—1932				January 20		8.6	17
February 8	1.48	(9.6)	36	January 26	1.13	9.1	42
February 10		(8.9)	70	January 27		13.4	40
February 12	1.46	(10.5)	50	March 9	.99	8.2	17
February 16		(6.5)	80				
February 17	1.45	(8.1)	80	I—1934			
February 18		(10.6)	44	March 14	1.23	(9.1)	44
February 19	1.44	(7.5)	60				
D—1933				L—1935			
January 6	1.05	10.6	60	January 10	1.57	(12.4)	17
January 17		9.1	48	January 15	1.45	8.0	44
January 30	.99	6.8	46	February 14	1.39	12.4	32
January 31		7.5	22				
February 1	.96	8.5	35	M—1935			
February 2		15.1	36	July 30	1.30	(12.5)	27
February 8		9.0	34	July 31	1.29	(12.5)	16
February 10	.96	4.6	60	August 1	1.28	8.9	21
E—1933				August 2	1.27	9.9	33
March 2	1.79	6.9	35	August 3		(10.5)	23
March 3		6.1	35	August 5	1.26	(10.4)	60
March 6	1.76	5.6	41	3—1936			
March 7		5.4	30	December 28	1.90	18.6	80
March 8		6.8	22				
March 13	1.73	6.8	23	26—1937			
March 14		5.9	13	January 27	1.44	5.4	33
March 17		(8.4)	32	January 28		5.2	50
March 18		(8.9)	15	January 29		10.9	32
				January 30		10.9	29
				February 9	1.35	11.3	26
				February 10		10.5	32

¹ Values in parentheses are environmental temperatures. The rectal temperature, although not measured, was undoubtedly only slightly above that of the environment (see page 146).

² 1932.

many relatively large marmots hibernated, it so happened that either they were not in deepest hibernation on the days when metabolism measurements were made, or when they were in this condition the press of other researches prohibited observation on them.

The rectal temperatures of A, B, and I were not measured as we did not

wish to disturb the hibernating animal and to run the risk of waking it by inserting the thermometer. With those marmots in which the thermo-junction could be maintained a continuous study of the rectal temperature was possible. The tendency of the animal to wake up and dislodge the thermometer, frequently destroying it, which meant the disturbance of inserting another thermometer, prevented many observations. As the rectal temperature of the hibernating marmot is always about 1° to 3° above the environmental temperature (see page 146), in those instances where the rectal temperature was not measured the environmental temperature has been recorded in parentheses in table 40 as a rough indication of the probable cell temperature. The minimum heat production occasionally was measured for only one hour, but more frequently for four or five hours and in some cases for seven or eight hours. The average values shown in the table are in most cases based on two or more periods of measurement.

In this discussion emphasis is laid on the actual level to which the energy metabolism of these animals could descend. As many of these records were obtained while the animal was inside a metallic chamber and hence not visible, simultaneous records of the respiration rates were not obtainable. In the instances where they were counted, notably with No. 3 and No. 26, the respiration rate was approximately 1 per minute. In fact, the rates of No. 26 are quite in line with our detailed observations on respiration rate during hibernation, ranging only from 0.3 to 4 per minute. On December 28, No. 3 had a rate of 4 per minute.

COMPARISON OF BODY TEMPERATURE AND HEAT PRODUCTION OF THE HIBERNATING MARMOT

The rectal temperatures of these hibernating marmots, for the most part, ranged between 5° and 12° C. Many of the rectal temperature observations were made when the marmot was not subjected to respiratory studies. Comments already have been made upon the possible depth to which the rectal temperature may descend and the animal still survive. The simultaneous measurements of the metabolism and the rectal temperature permit direct comparisons. Although the heat production of warm-blooded animals is not usually accompanied by any significant alterations in body temperature, it is maintained that even slight differences in rectal temperature are reflected in the heat production. With the cold-blooded animals, on the other hand, the heat production is almost directly proportional to the rectal temperature. Without doubt the hibernating animal lies somewhere between these two extremes. With the animal entering hibernation there is a general tendency for the heat production to be lower, the lower the rectal temperature, although this correlation is not sufficiently accurate to accept the rectal temperature as an index of the metabolic level. In deep hibernation the marmot more directly resembles the cold-blooded animal and the tendency indicated in the transitional phase may be for the moment assumed to develop into a fixed relationship. To what extent this relationship is fixed

and to what extent it is variable can be noted from the data in table 40. Because the hibernating marmot must be considered as bordering at least on the cold-blooded state, simultaneous discussion of the rectal temperature and the metabolic level must be given. Consideration of the possible correlation of the metabolism with the respiration rate and the heart rate will be left until later.

The lowest rectal temperatures appearing in table 40 are close to 5°, and are noted with animals D, E, and No. 26. The highest rectal temperature when the animals were considered to be in deep hibernation was that of No. 3 on December 28, namely, 18.6° C. The finding of a close correlation between the rectal temperature and the metabolism of the cold-blooded animals would suggest that there might be a similar relationship with hibernating animals, but this is far from the case. At a rectal temperature, for example, of 10° to 11° C., marmot D on January 6 had a minimum heat production of 60 calories, whereas between these limits No. 26 on January 30 had a heat production half this, 30 calories. At a rectal temperature of 5° to 6° marmot E's metabolism on March 14 was at its lowest level, 13 calories, but on another day, March 6, at this same rectal temperature its metabolism was 41 calories, or more than three times the other value. No. 26 on January 28 had a rectal temperature of 5.2° and a heat production of 50 calories. Thus there are great differences in the heat production of the hibernating marmot, even with the same rectal temperature.

At a given heat production there is also a wide range of rectal temperatures. No. D on January 6 had a metabolism of 60 calories and a rectal temperature of 10.6° C., but on February 10 when its heat production was the same, its rectal temperature was 4.6° C. At a body temperature half way between 5° and 11°, its heat production was only 22 calories. No. 3 had a high rectal temperature of 18.6° and a low heat production of 80 calories. Marmot D had a temperature of 15.1° and a heat production of only 36 calories. These values may be contrasted with the 50 calories found with No. 26 at a temperature of 5.2°, and the 60 calories with D when its rectal temperature was 4.6°. In these studies the marmots were in a static condition. They had had the rectal temperatures shown in table 40 for some time, and the measurements were not made in a transition phase. These cases noted in table 40 emphasize the lack of a relationship (such as obtains in cold-blooded animals) between the rectal temperature and the heat production of the marmot when in deep hibernation.

Table 41 shows a still more striking picture. Thus, marmot D on January 6, at a rectal temperature of 9.2° C., had a heat production of 140 calories. During the following 6 hours its temperature increased approximately 1.5°, but the heat production fell. The rise in rectal temperature was accompanied by a rise in environmental temperature. This same trend is shown on January 17. There are instances in table 41 where the environmental temperature is above the rectal temperature. This is because a transitory state is in progress, and the rectal temperature lags

behind the rapidly rising environmental temperature. On February 7 the rectal temperature was as low as 3.3° and the heat production was at the surprisingly high level of 240 calories. Inasmuch as the basal heat production of the non-hibernating marmot is 400 calories and the rectal tempera-

TABLE 41—*Reaction of heat production of the hibernating marmot to cold environments*

Marmot and date	Period		Temperature		Heat pro- duction per $10^{w^{2/3}}$ per 24 hours
	Time	Length	Environ- mental	Rectal ¹	
1953		min.	°C.	°C.	cal.
Marmot D					
Jan. 6	9.30 a.m.	135	9.7	9.2	140
	11.45 a.m.	135	10.0	...	80
	2.00 p.m.	140	10.3	10.6	60
Jan. 17	10.00 a.m.	120	8.3	8.0	140
	12.00 noon	120	8.5	...	100
	2.00 p.m.	126	9.0	9.1	48
Feb. 7	9.30 a.m.	22	2.0	3.3	240
	10.30 a.m.	22	1.6	3.5	150
	11.15 a.m.	22	2.0	3.5	150
	11.50 a.m.	35	2.4	3.8	130
	1.33 p.m.	35	3.0	3.9	120
	2.45 p.m.	45	4.0	5.0	120
Feb. 10	11.02 a.m.	44	0.9	4.1	130
	12.42 p.m.	85	3.0	4.5	70
	2.11 p.m.	80	2.2	4.6	70
	3.36 p.m.	55	2.6	4.7	47
Marmot E					
March 6	10.03 a.m.	12	2.5	5.0	150
	10.56 a.m.	20	2.4	5.6	60
	11.33 a.m.	25	2.5	5.7	60
	12.21 p.m.	25	2.7	5.7	37
	1.13 p.m.	26	3.4	5.7	44
	2.01 p.m.	25	3.7	5.6	41
	2.45 p.m.	30	4.3	5.6	36
	3.30 p.m.	30	4.5	5.6	47
Marmot 26					
Feb. 4 ²	9.45 a.m.	20	1.8	5.5	340
	10.05 a.m.	20	1.8	5.5	340
	10.25 a.m.	20	1.9	5.6	340
	10.45 a.m.	30	1.9	5.6	350
	11.15 a.m.	31	1.8	5.7	340
	11.46 a.m.	29	2.3	5.9	290
	12.15 p.m.	30	5.4	6.0	230
	12.45 p.m.	30	9.9	6.1	150

¹ Under conditions of rising environmental temperature the rectal temperature also rises but more slowly than the environmental temperature. This frequently causes the rectal temperature to be lower than the environmental temperature.

² 1937.

ture at the basal level is 37°, this instance of a rectal temperature 34° below the basal level and a heat production only 50 per cent lower is truly remarkable. Here as the body temperature rose the heat production was halved. On February 10 the heat production of D fell to almost one-third the initial

level, and the rectal temperature increased but 0.6° C. This is similar to the trend shown in the experiment of January 17 on this animal, but in that case the rise in rectal temperature was about 1° C. With E the heat production decreased from 150 to 36 calories, with less than a one-degree increase in rectal temperature. In these last two experiments the environmental temperature rose at least 2°. No. 26 with a relatively low body temperature of 5.5° had a heat production of 340 calories, *i.e.*, as high as some non-hibernating marmots measured in the basal condition. The initial environmental temperature here was lower than the rectal temperature by almost 4°. Increasing the environmental temperature to 10° resulted in a rise of 0.6° in rectal temperature and a 56 per cent decrease in the heat production, *i.e.*, to 150 calories. According to table 40 marmot No. 26 at a rectal temperature of 5.4° had a heat production as low as 33 calories, but in that case the environmental temperature of 3.6° was much nearer the rectal temperature.

In each instance mentioned there was an increasing environmental temperature, which resulted not only in a slight increase in rectal temperature but a decrease in heat production. Thus it is evident again that the minimum heat production is by no means always accompanied by the lowest rectal temperature, and that raising the environmental temperature often lowers the heat production. These findings would indicate the retention of a vestige of the chemical regulation. In other words, these marmots even though in hibernation have a chemical heat regulation at a cold environment. The fourfold increase or more in metabolism of the hibernating marmot is not accompanied by visible external muscular movements. In hibernation there is nothing which resembles a muscular movement for hours, if not days. Frequently the animals are not able to make any body movements, except a major movement which under these conditions is very slow. If one were to pick the animal up, one would find it cold and stiff. Under these conditions it requires considerable effort on the part of the experimenter to alter the relative position of the limbs. Thus this increased heat production cannot be attributed to muscular movement but must be due to an internal cell stimulus.

This analysis gives a clear indication of what one might term a pure chemical regulation. Rubner contended that such a phenomenon is a true chemical regulation when practically, if not wholly, devoid of any physical movements. Others maintain that a chemical regulation or increase in metabolism unaccompanied by muscular activity cannot be found. With our marmots when in profound lethargy 6-, 8-, or 10-fold differences in metabolism were frequently noted, along with extraordinarily low rectal and environmental temperatures. This feature of this study is perhaps most pronounced in emphasizing the existence of the pure chemical regulation. It is strongly reminiscent of the experience of the Nutrition Laboratory with a professional artist's model who, by her training, was accustomed to posing nude and, owing to the minimum of heat in artists' studios, was frequently exposed to

very cold rooms. In our studies of her metabolism in the laboratory under controlled conditions, it was found that she could lie nude in a room at 11° for half to three-quarters of an hour without any alteration in the metabolic level over that when she was clothed. There was no visible movement and frequently the subject was asleep. Only after 30 to 45 minutes was a rise in oxygen consumption noted.²⁴ This was not associated with muscular movements and thus gave clear evidence that at least with humans the pure chemical regulation can take place. Since in hibernation there is nothing which resembles a muscular movement except when the animal is waking or entering hibernation, it would seem that here also is an instance of pure chemical heat regulation.

MINIMUM HEAT PRODUCTION OF THE HIBERNATING MARMOT

The lowest 24-hour heat production recorded in table 40 is that of E on March 14, namely, 13 calories per $10w^{\frac{3}{4}}$. This same animal on March 18 also had a heat production of 15 calories. Marmot M on July 31 reached the low level of 16 calories. On two days, January 20 and March 9, F produced only 17 calories, a level also noted on one day, January 10, with L. Thus with four marmots on at least one day or more values of 17 calories or below were found for one hour or over. Therefore, 17 calories, approximately the level found by Dontcheff and Kayser,²⁵ can probably be stated as being the lowest level of heat production of these animals. If values up to 26 calories are included, it is found that in addition to the several values for the marmots already mentioned, that for D on January 31, 22 calories, would come within this limit. No. 26 on February 9 also reached this level. This group of values of 26 calories and under now includes six animals. Among all the 52 values obtained, there are 31 below 40 calories that average 27 calories. Thus 27 calories would be an *average minimum* value and 17 calories could be accepted as the *absolute minimum* attainable by the marmot.

COMPARISON OF METABOLISM OF MARMOT WHEN NON-HIBERNATING AND WHEN HIBERNATING AT A LOW ENVIRONMENTAL TEMPERATURE

Marmot M on July 17 was measured at an environmental temperature of approximately 9° C. The animal on this date had not hibernated and was awake. Five periods of measurement without gross muscular activity indicated a heat production per $10w^{\frac{3}{4}}$ of 1500 calories, a direct effect of the cold. Two weeks later, on August 1, at the same environmental temperature, this animal in deep hibernation had a heat production of 21 calories, and at a chamber temperature 4° higher on July 31 a heat production of 16 calories. Thus, the animal had been able to have a metabolism on one day practically 100 times that found two weeks later, in both cases without appreciable muscular movement and at essentially the same environmental temperature,

²⁴ Benedict, F. G., Bull. Soc. Sci. d'Hygiène Alimen., 1927, 15, p. 172.

²⁵ Dontcheff, L., and C. Kayser, Compt. Rend. Soc. de Biol., 1935, 119, p. 565.

though in the latter case the *rectal* temperature was probably 25° lower than in the former case.

HEAT PRODUCTION UNDER THE INFLUENCE OF NEMBUTAL

As has been shown in the previous discussion of the rectal temperature, the heart rate, and the respiration rate, marmots cooled under nembutal

TABLE 42—*Metabolism of marmot No. 24, under nembutal, at low temperatures*
(Body weight, 1.8 kg.)

Date	Time	Rate per minute		Rectal temp. ¹	Heat production per 10w ^{2/3} per 24 hrs.
		Heart	Respiration		
<i>1937</i>					
Jan. 11	2.00 p.m.	15	5	12.0	29
	2.30 "	15	11.6	28
	3.01 "	15	5	11.7	28
	3.31 "	15	11.6	28
	4.00 "	15	5	11.7	28
Jan. 12	10.30 a.m.	17	6	12.1	29
	11.00 "	17	12.1	34
	3.42 p.m.	17	6	12.0	31
	4.12 "	17	8	12.0	35
Jan. 13	10.45 a.m.	17	6	12.4	33
	11.20 "	17	7	12.3	32
Jan. 14	10.26 a.m.	19	8	11.8	38
	11.01 "	19	8	11.8	37
	3.40 p.m.	17	7	11.4	34
	4.15 "	16	7	11.3	32
	10.30 "	17	8	11.9	34
	11.05 "	17	8	11.6	33
Jan. 15	10.35 a.m.	19	7	12.1	39
	11.55 "	19	7	12.0	37
	1.25 p.m.	19	8	12.1	40
	3.03 "	20	12.0	42
Jan. 16	9.20 a.m.	19	5	12.9	35
	10.30 "	12.7	36
Jan. 18	8.40 a.m.	21	9	11.9	33
	9.18 "	20	9	12.0	33
	10.23 "	21	6	12.6	34
Jan. 19	8.16 a.m.	19	4	11.1	27

¹ Environmental temperature averaged 1° below the rectal temperature.

did not attain the minimum levels of these functions noted in animals normally hibernating. As the heat production of marmots when drugged with nembutal²⁶ and cooled was measured, it is important to note how closely the metabolism under such conditions approached the minimum normal hibernating level. No. 2, under this drug, reached a level of 35 to 37 calories per 10w^{2/3} on at least three days. Indeed, on one day a measurement for 40

²⁶ It has been shown that nembutal itself does not lower the basal metabolism (Cavett, J. W., Proc. Amer. Soc. Biol. Chemists, 1937, 8, p. xvii).

minutes gave a value of 26 calories, when the rectal temperature was 13.8° C. As the animal died on this day, the significance of this measurement is debatable. No. 1, with a rectal temperature of 16.5° C., reached a level of 37 calories, and No. 4 with a rectal temperature of 13.0° C., reached 40 calories.

In addition to these observations, a striking experiment was made with No. 24, which was maintained under the influence of nembutal for 12 days. During the entire time heart rate, rectal temperature, and respiration rate remained reasonably uniform. (See table 42.) The heat production was measured on only eight days. It was first determined on January 11 (4 days after the marmot was initially drugged on January 7) and the level for over $2\frac{1}{2}$ hours was uniform at 28 calories. The following day, an hour's measurement in the morning showed a slightly higher level of 32 calories and in the afternoon of 33 calories. This level was also found on January 13. On January 14 the marmot was measured for about an hour at three different times during the day. These three measurements gave uniform results, agreeing with the previous measurements. On January 15 the heat production was the highest measured in this series, being 42 calories. The other measurements in this series were all at about the 30-calorie level. The range for the entire series from 27 calories on January 19 to 42 calories on January 15 (a difference of only 15 calories) shows remarkable uniformity in the metabolism, when one considers the lability of the metabolism that may obtain in normal hibernation. As these measurements were made at various times during the day and the heat production was always found to be 42 calories or below, the assumption is justified that the metabolic level was never above this. The average for the entire series, based upon well-agreeing period values, is 33 calories. This is not far from the average value of 27 calories per $10w^{\frac{3}{4}}$ found with the normal marmot in deep hibernation.

COMPARISON OF THE METABOLISM OF THE HIBERNATING MARMOT WITH THAT OF COLD-BLOODED ANIMALS

We now resume our quest for a bridge between the warm- and the cold-blooded animal. The Nutrition Laboratory's survey of the larger cold-blooded animals has shown that when the cold-blooded animals were measured at different temperatures, they had an increasing metabolism with increasing temperature. When the cold-blooded animal was warmed to the temperature of the normal warm-blooded animal, that is, 37° C., its metabolism, however, never reached the level of the warm-blooded animal. Thus at a temperature of 37° the heat production per $10w^{\frac{3}{4}}$ was 110 calories.²⁷ This is only about one-fourth of the value for the non-hibernating marmot and one-ninth of the general average figure of about 1000 calories per square meter of surface area commonly ascribed to warm-blooded animals. Our next objective is to determine how near the marmot in hibernation ap-

²⁷ Benedict, F. G., Carnegie Inst. Wash. Pub. No. 425, 1932, fig. 103, p. 472, and table 21, p. 473.

proaches the true cold-blooded animal. The rectal temperature of the marmot can decrease to 3°, possibly under, and still the animal will recover. The cold-blooded animal can recover after its temperature falls to 0° and possibly under, for there are reports of frozen fish that have been subsequently thawed out and found to have life. Marmots in hibernation have respiration rates of 0.2 per minute, which is even below the value of 2 per minute found with snakes. Thus, in many ways the hibernating marmot simulates the cold-blooded animal. In one respect, however, it is different. Its heat production even at the lowest level is always higher per $10w^{\frac{1}{2}}$ than that of the cold-blooded animal. A rectal or cell temperature of 9° would with the snake correspond to a metabolism of 8.0 calories per $10w^{\frac{1}{2}}$. When the hibernating marmot has a rectal temperature of essentially 9° (see table 40), its heat production is almost uniformly in the neighborhood of 25 to 30 calories per $10w^{\frac{1}{2}}$, which is at least three times greater than the metabolic level of 8.0 calories shown by the general curve for cold-blooded animals.

As a matter of fact, there were a number of instances where the hibernating animal had a heat production even lower than this, as low as 16 or 17 calories. Probably enough period measurements are available to state definitely that 17 calories is the lowest heat production of the hibernating marmot, although this is not necessarily always at the lowest rectal temperature. Marmots M and L had a metabolism as low as 16 and 17 calories, but unfortunately no rectal temperatures were taken. As the environmental temperature on these days was 12°, there is every reason to believe the rectal temperature was above this. In this case, if the hibernating marmot has a heat production of 17 calories at a rectal temperature of 12°, this would still place the marmot above the cold-blooded snake, for at 12° the heat production of cold-blooded animals is 11 calories. There is one instance with E on March 18 where the environmental temperature was 9° and hence the rectal temperature slightly higher, and the heat production was 15 calories, which approaches the 8 calories at 9° C. for the cold-blooded animal. The lowest metabolism, 13 calories with E on March 14, was at a rectal temperature of 5.9° C. At the same temperature the cold-blooded animal would produce 5 calories. Thus, even at the lowest level the heat production of the marmot is still at least twice that of the cold-blooded animal. These, however, are special cases, and in general it may be properly stated that the hibernating marmot's metabolism of 27 calories is three times that of the cold-blooded animal having the same rectal temperature.

These several comparisons between the metabolism of the marmot in deep hibernation and the metabolism of the cold-blooded animals (snakes) at identical rectal temperatures have been based upon the heat production per $10w^{\frac{1}{2}}$, the values for the marmot representing actual measurements and those for the cold-blooded animals being derived from a general curve based on actual measurements. Because of the low heat production prevailing with both groups of animals at rectal temperatures of from 5° to 10°, the ac-

tual differences in metabolic levels numerically do not seem to be very large. Thus the difference between 11 and 17 calories is numerically small, but percentagewise it is large. It is recognized that the comparison on this basis is open to the objection that whereas the marmots that were measured weighed approximately 1 to 2 kg., the snakes were for the most part heavier animals. Hence, as it is proved that the computation on the basis of $10w^{\frac{3}{4}}$ equalizes the differences in size of cold-blooded animals far better than it does in the case of warm-blooded animals, this type of comparison may not seem justifiable. There are not sufficient data on snakes of precisely the same size as the marmots to permit extensive calculations on the basis of the heat production per kilogram. Such data as are available indicate that the general statement is correct that the heat production of the hibernating marmot does not descend to the metabolic level of the cold-blooded snake at the same cell temperature.

Among the gopher snakes that were studied the smallest single snake weighed about 1.92 kg., that is, not far from the weights of most of our marmots reported in table 40. The heat production of snakes of this size per kilogram of body weight per 24 hours was not measured at a temperature below 16.5° C. At this temperature the heat production of the snake was 1.2 calories per kilogram. From the general course of the curve it can be readily computed that at 12° the metabolism of the gopher snake would be considerably below 1 calorie per kilogram. With the marmot, on the other hand, the lowest values found are 1.3 to 1.5 calories per kilogram, or again nearly three times the probable value for the snake measured at a corresponding rectal temperature.

No devotee of the surface area concept can take exception to the calculations given above, in which the factor K in the surface area formula $S = K \times w^{\frac{3}{4}}$ has been used as 10 in all instances. The value of 18.6 given by Rubner as the K for the snake is twice 9.3, the best value of K for the marmot. If these values are used to calculate the surface area, the snake will have a surface area twice that of the marmot, provided it is of essentially the same weight. Since at the same cell temperature the snake has a total heat production less than that of the marmot, use of the factors 18.6 and 9.3 would result in an even greater difference in the calculations of heat production per square meter of surface area than exists in the calculations per $10w^{\frac{3}{4}}$.

Prior to this study the difference in the metabolism of the hibernating animal and the cold-blooded animal would have been explained as simply indicating a greater cell activity of the hibernating animal, because it has a larger blood supply and therefore can live at a higher tempo than the cold-blooded animal. But our research has emphasized how frequently these hibernating animals, even with a very low rectal temperature, may have a high heat production up to 300 calories or more per $10w^{\frac{3}{4}}$. Thus the reaction of an increased metabolism to the cold environment, which is so pronounced with the non-hibernating marmot and which is not found with the cold-blooded animal, is accentuated in the hibernating marmot, even at a

very low rectal temperature. The absence of correlation between the total heat production and the rectal temperature of the hibernating marmot is in striking contrast to the picture shown by the cold-blooded animals, for the minimum heat production of the former is frequently found at a rectal temperature of 10° or over and at times the lowest body temperatures are accompanied by a relatively high metabolism. One must here consider that in one case the metabolism is affected by the rectal (cell) temperature itself and in the second case the metabolism is affected by the environmental temperature. The rectal temperature of the cold-blooded animal follows the environmental temperature almost perfectly and very rapidly. With the hibernating marmot the situation may be somewhat different. In the first place, the rectal temperature of the hibernating marmot is invariably somewhat above the environmental temperature, whereas that of the cold-blooded animal is usually below. Secondly, even when it has a very low rectal temperature, the hibernating animal still reacts *at times* to low environmental temperatures in the same general manner as does the non-hibernating animal. To be sure, this reaction with the non-hibernating marmot is frequently transitory, and under the same environmental conditions the animal may quickly go into true hibernation with a rapidly decreasing heat production. Oftentimes a vestige of the chemical regulation comes into play with these marmots, even at low temperatures. Whether chemical regulation in hibernation is less at the lower temperature is not evident from the data at hand. However, it is clear that cell temperature is not a dominating factor in heat production.

For our bridge between the cold-blooded and the warm-blooded animals the marmot does not connect the two abutments smoothly, for at the warm-blooded or upper side the metabolism of the non-hibernating marmot is, on the whole, below that of the average warm-blooded animal, and at the lower or cold-blooded side the marmot has a heat production per 10w³ higher than that of the cold-blooded animal, although it can attain the same cell temperature common to cold-blooded animals. However, as its metabolism approximates both of these levels, the marmot may be considered to represent most nearly a well-constructed bridge between the two great groups of cold-blooded and warm-blooded animals.

CORRELATION OF VARIOUS PHYSIOLOGICAL FUNCTIONS

A study of the relation of the various functions to each other is important not only to establish a correlation, but it may be valuable as a basis to form some concept as to the mechanism which governs these physiological processes. The study of the rectal temperature has demonstrated that in true hibernation the cell temperature is always slightly above the environment, usually 1° to 3° C. Therefore, the criteria of hibernation involves the rectal temperature. That there is no relation of the rectal temperature to the respiration rate, the heart rate, or the heat production has already been shown, and the absence of a relation is strikingly emphasized in table 43 where

the heart and respiration rates and heat production are classified according to the ascending levels of the rectal temperature. Perhaps the most signifi-

TABLE 43—*Comparison of heart rate, respiration rate, and heat production at various levels of rectal temperature*

Marmot No. 26

Date	Level of rectal temperature	Rate per minute		Heat production per $10\text{w}^{1/2}$ per 24 hours cal.
		Heart	Respiration	
1937 Jan. 28	3.0	1 1 1 1 1	46 48 50
	
		0.7 0.7 2	33 32 70
		20	340
		20 21 21 19 37 22 18	4 4 4 4 4 4 3	340 340 350 340 290 230
Jan. 27	5.0	0.7 0.7 2	33 32 70
" 28
Feb. 4
" 4	6.0	10	2	150
		15
Jan. 28	7.0	1	45
Feb. 4	20	3
" 16	5	25
Jan. 28	8.0	0.3	38
Feb. 16	4	0.6	27
Feb. 16	9.0	5	25
Jan. 29	10.0	1	33
Feb. 9	5	0.3	25
" 10	5	0.8	31
" 16	5	32
		4	34
Jan. 30	11.0	31
		0.4	28
Feb. 9	5	0.3
" 10	4	27
		6	43
" 16	8	60
		5	0.8	29
" 16	5	28
		5	1	29
" 16	5	30
		5	1	29
" 16	5	24

cant fact brought out by this table is that at rectal temperatures in the vicinity of 10° the heat production is usually low. A general survey of

all the data substantiates this finding. This is readily explained when one considers that at 10° there is probably no need of a chemical heat regulation

TABLE 44—Comparison of heart rate, respiration rate, and rectal temperature at various levels of heat production
Marmot No. 26

Date	Level of heat pro- duction per $10w^{2/3}$ per 24 hours	Rectal temperature	Rate per minute	
			Heart	Respiration
1937				
Feb. 9	cal.			
" 16	20-25	11.4 9.5 8.0 7.3	5 4 4 5	0.3
Jan. 30	26-30	10.9	0.4
Feb. 9		11.1	4
" 10		11.4 10.5 11.0	5 5 5	0.3 0.8
" 16		11.2 11.3 11.2 11.2 11.3 10.1 8.5	4 5 5 5 5 4 4	1 1 0.6
Jan. 27	31-35	5.7 5.4 5.2	0.7 0.7 0.7
" 29		10.8	1
Feb. 10		10.4 10.3 10.6 10.4 10.4	5 6 6 5 5	0.8 1
Jan. 28	36-45	7.7 8.2	1 0.3
Feb. 10		11.2	6
Jan. 28	46-65	3.8 3.7 3.6 5.4	1 1 1 2
Feb. 4	100-200	6.8	10	2
Feb. 4	201-300	5.8 5.9	18 22	3 4
Feb. 4	301-400	5.6 5.5 5.6 5.6 5.7	20 21 21 19 37	4 4 4 4 4

to prevent the animal from freezing, a need which may be present at lower temperatures. Unfortunately the experiments in which the heart rate, the respiration rate, the rectal temperature and the heat production are

simultaneously measured, are confined to one animal (No. 26), but since the measures were taken on several days and the animal was awake between some of these experiments, the picture shown is probably representative.

By classifying the functions for each of the various periods of measurement on the several days according to the various increasing levels of the heat production, as has been done in table 44, it is obvious that, with the excep-

TABLE 45—Comparison of heart rate, respiration rate, and heat production at various levels of rectal temperature of marmots under nembutal

Marmot	Date	Level of rectal temperature	Rate per minute		Heat production per $10w^{2/3}$ per 24 hours
			Heart	Respiration	
24	1937	°C.			
	Jan. 11	12.0	15	5	28
	" 12		17	6	32
	" 13		17	7	33
	" 14		19	8	35
	" 15		20	7	40
	" 16		19	5	36
	" 18		21	8	34
2	May 28		18	3	39
	" 29		10	4	37
Average			18	6	35
21	May 24	14.0	21	4	
18	¹ Dec. 7	16.0	27	4	80
1	May 22		21	3	33
2	May 24		23	3	33
	" 27		26	2	70
Average			24	3	54
18	¹ Dec. 7	18.0	31	7	100
1	May 22		26	3	39
2	" 24		50	3	80
	" 27		28	1	80
	" 30		75	3	120
	June 1		74	3	110
Average			47	3	88
18	¹ Dec. 7	20.0	31	8	130
1	May 22		33	2	50
2	" 24		46	5	150
	" 27		36	100
	June 1		100	6	160
Average	.		31	5	118

¹ 1936.

tion of the rectal temperature, the functions bear a general relationship to each other. Specific examination of the respiration rates for a heat production of from 26 to 30 calories shows wide variations of from 0.3 to 1 per minute, the highest values more than three times the minimum. Again at the 31- to 35-calorie level and the 36- to 45-calorie level there are likewise large variations percentagewise, for a given caloric level. Since the minimum of

0.3 per minute in this series is found at the 36- to 45-calorie level as well as at the lowest level, there is no indication that the respiration rate is definitely associated with the heat production from 20 to 45 calories.

At the highest heat levels the respiration rate is higher, so that in general a higher respiration rate accompanies a higher heat production but not in a direct proportion.

An analysis of the heart rate at the various levels of heat production reveals a much greater uniformity at a given level. The range is one count at each of the three lowest heat levels. At each higher caloric level the heart rate is, in general, higher, the whole picture being very consistent. Therefore, the heart rate does have a fairly definite correlation with the heat production.

For animals cooled and under nembutal the general picture of the various functions of heart rate, respiration rate, and heat production are shown in table 45. In general as the rectal temperature rises the heart rate is faster, but in the case of the respiration rate the lowest values are not found at the lowest rectal temperature. This is in accordance with some experiments on the normal, hibernating animals, where the respiration rate decreased as the rectal temperature increased. At the lower rectal temperature level the heart rate is always low and the variation is not great, but at the higher level of 20° C., much greater variability is found. This is also true at 18° C. The average heat production is higher at each higher level of rectal temperature.

Conclusions. Under nembutal the heart rate and the heat production increase with the rectal temperature, but there is no close correlation between the respiration rate and these functions.

In normal hibernation the heat production, the heart rate, and the respiration rate have a general relationship, but the rectal temperature has no close correlation. The heart rate, of all the functions, has the closest correlation to the heat production.

PHYSIOLOGY OF THE MARMOT WHEN AWAKENING FROM HIBERNATION

The process of entering hibernation is characterized by a partial descent into hibernation, coming out, descending again, perhaps a little deeper, again coming out and finally going into deep hibernation. Many references have been made to the susceptibility of the animal entering hibernation to various stimuli which cause it to come out of hibernation or out of partial hibernation. The stimulus of handling has been shown to have a very definite effect, usually causing the animal to awaken fully, and even noise has been shown to have an awakening influence. Although we are not concerned as to whether animals remained awake following the process of awakening from hibernation, the level from which the start is made is important. Our real objective is to study the various physiological processes during awakening, starting from a stationary hibernating level. From our study of animals in the non-hibernating condition and from the complete hibernation study it is known at what levels the animal will start, and to what levels it ought to rise. As we have already established the start and end, the question arises, will the awakening animal over-shoot these levels, or will the process be very gradual, only approaching the limit after an extended period of time.

STIMULI TO WAKING

Contrary to common opinion, these animals when in deep hibernation are not so unconscious as they seem. We have found them very sensitive to external and internal stimulation, and that all the extraordinary phenomena noticed with these animals in passing from the waking condition to hibernation, phenomena which initially occupy several days, if not weeks, are intensified in a relatively short time, usually a few hours, when the reverse takes place, that is, when the animals pass from hibernation to being fully awake.

There are several possible causes for waking, external or internal, each of which may be characterized by some particular phenomena either as to the intensity or course of the process, the time required to complete the process, or the levels reached. It is possible that the various physiological functions will react differently to these several stimuli. Thus the process of waking from hibernation has important contributions to physiology.

External stimulation may take the form of handling, insertion of a thermometer, the possibility of intense cold, and likewise heat or warmth. To make observations on animals in hibernation, handling is usually necessary, such as in transporting the animal from its cage to the chamber or the intimate handling for the insertion of a rectal thermometer and still greater contact to attach electrodes to the feet. It was only infrequently that all these operations could be carried out without causing the animal to waken.

Pflüger¹ maintained that there is a protective mechanism present in the marmot which, when it is subjected to extremely cold environments, such as freezing temperature or below, prevents freezing by causing the marmot to awake. We were never successful in waking our animals by subjecting them to severe cold. Several animals were subjected to room temperatures as low as -2° or -3° C., but no animal was wakened at will by such exposure. Marmot I entered hibernation while kept at essentially a freezing environmental temperature. Prior to February 8 it had not hibernated. On February 8, at an environmental temperature of 2° , it had a rectal temperature of 36° C., whereas on February 9 and 10 when room temperatures as low as -1° existed (as can be seen in table 20), it had a rectal temperature of 18° and 25° , respectively. Animal K on February 8 had a rectal temperature of 5.6° and on the 9th and 10th its rectal temperature was between 11° and 12° . It is clear that the low temperature did not cause this animal to awaken, although frequently the cold did result in a relatively high heat production. Indeed, when the environment, in which an animal with a high heat production was being maintained, was warmed, there was an actual lowering in the heat production as the temperature rose. At times this warming was followed by the complete waking of the animal, and the low periods of heat production were immediately followed by an excessively rapid heat production characteristic of the waking from hibernation. At times animals did wake at cold temperatures, but since there were semi-periodic waking times it is not clearly shown that the waking did not coincide with such a period. There is no evidence that room temperatures as low as 5° resulted in any tendency for the animals to waken. From the study in hibernation it was noted that at the lowest environmental temperatures frequently there was a heat production notably higher than the minimum observed in deep hibernation. The chemical heat regulation, which was clearly shown at times in the hibernating period, may be called a tendency in this direction. The fact remains that we have no definite cases where animals were obviously wakened by subjecting them to cold temperatures.

Some animals that were maintained under uniform environmental conditions wakened without external stimulation. This would be the result of an internal stimulation, such as the possible necessity for urination or defecation. In the course of our studies this normal waking due to internal stimulus, already noted by many others, was found frequently, thus showing that the marmots may not stay for a long time in deep hibernation.

When we desired to waken our animals and follow experimentally the phenomena, two methods were used, one, warming the chamber in which the marmot was placed to the zone of thermic neutrality, or to the non-hibernating body temperature level. Another method, that of faradic shock, used by Saissy,² was employed in one instance. These methods were always

¹ Pflüger, E., Arch. f. d. ges. Physiol., 1875, **10**, p. 475.

² Saissy, J. A., Mém. de l'Acad. de Turin, 1811, pp. 1-24. (Cited by Polimanti, O., *Il Letargo*, Roma, 1912.)

productive of the waking phenomena and will be more fully discussed in connection with the protocols.

During the course of the transition, occupying as it does but two or three hours, no significant changes in body weight are to be expected. An animal in this stage is so valuable, so to speak, from the physiological standpoint that emphasis is laid upon the measurement of the other physiological factors during this time with practically no regard to body weight. The same may be said with regard to the insensible perspiration. It is perfectly conceivable that if an animal were left on the balance and weighed during waking, actual changes in weight could be observed. Since, however, the animal would normally have increased its body temperature enormously, one can think of several technical difficulties in weighing. The body would be continually warming in its internal temperature, causing ascending convection currents of the air from the animal's body, and secondly, the warming of the cold body and cold fur would change the hygroscopic conditions and undoubtedly humidity changes would need to be considered. Consequently these tests did not seem to us desirable and were not made.

RECTAL TEMPERATURE DURING WAKING

One of the factors noted in many instances when the marmot was waking from hibernation was the course of the rectal temperature. In hibernation the rectal temperature has been shown to be only slightly above the environmental temperature, descending at times to a level below 5°, and nearly always being 12° or below when at a constant level. When the animal wakes it has a goal of 36° or 37° C. to attain.

The changes in rectal temperature when the animals start to wake up are no less than astounding, as clearly shown by Dubois.³ They are characterized, in the first place, by very great increases, and in the second place, by great rapidity. Since the initial temperature at which the animals start may vary, although it is usually not far from 10°, and the final temperature is in the neighborhood of 36°, the entire rise is approximately 25°C. (See figure 10.) Even greater ranges in temperature have been observed, notably with animal E on February 20. This animal when first measured had the very low rectal temperature of 5.2° C. The room temperature evidently had been as low as or lower than this throughout the night, and had already commenced to rise in the morning and rose throughout the day, reaching a maximum of 10°. As has been pointed out in an earlier section, the body temperature of the marmot tends to follow changes in room temperature, but always lags behind the room temperature, whether it is rising or falling. This case illustrates it perfectly, for at first it took nearly 3½ hours for the temperature of this animal to get up to its normal height in hibernation, which would be 11° for an environmental temperature of 10°. Thereafter, evidently due to a stimulus to waking which appeared at this time, the animal started its true course of temperature rise incidental to

³ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, plate 3, p. 122.

waking and we have in a little more than $1\frac{1}{2}$ hours a rise from 10° to 37° , or a 27° rise, but the entire temperature rise from 5.2° in the morning to 37.6° , took place in 6 hours. With marmot E there is a great diversity in the course of the rapidity of these temperature rises, for on January 24 the animal began in the morning with a rectal temperature of 11.0° and had a steady rise in temperature, with a sharp break at about 20° , not noted on February 20.

On the other hand, animal F subjected to the same environmental situation, that is, an environmental temperature of about $9^{\circ}\text{ C}.$ on the same day, showed a much more rapid rise, for the rise from 11° to 36° was in a little over $1\frac{1}{4}$ hours. Finally, as an illustration of an intermediate type, we have animal No. 26, which was in the chamber immersed in a water bath. The

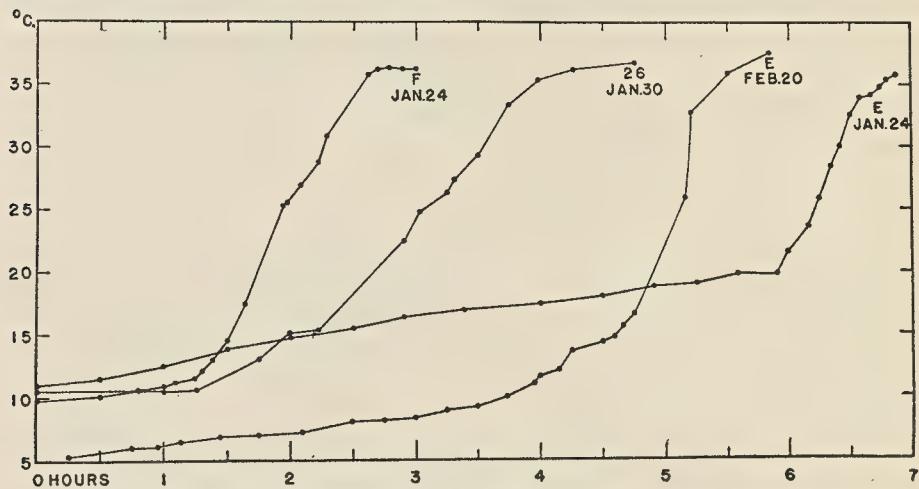


FIG. 10—RECTAL TEMPERATURE PLOTTED AGAINST TIME, FOR MARMOTS WAKING FROM HIBERNATION

Environmental temperature for experiments on January 24 and February 20 was below $12^{\circ}\text{ C}.$ For experiment of January 30, environmental temperature was raised to about 32° at 2-hour mark; animal handled at $1\frac{1}{4}$ hours. Other animals wakened due to internal stimulus.

animal was waked both by being handled and by being subjected to a warm environmental temperature of 32° . Under these conditions the temperature rose from 10.7° to 36° in about three and one-half hours. The striking point of this last experiment is that although this animal was very much favored by being in a respiration chamber immersed in warm water, the rise in temperature was, if anything, a little slower than in the case of E on February 20 and in the case of F on January 24, but far more rapid than with E on January 24, emphasizing the considerable irregularity in the rate at which these temperature rises take place. Although it is true that with animals E and F on February 20 and January 24, respectively, there is a similarity in the curves from 20° up to 36° , the irregularity with animal No. 26 and likewise with E on January 24 shows that there is no regular rule followed and that there is no general trend for any special condition.

One particularly striking point is the tremendous change which can occur in a very short time after the warming-up process is well under way. Between temperatures of 20° to 35° we frequently find enormous changes in rectal temperature in a matter of minutes. Animal E on January 31 raised its body temperature from 29.3° to 35.3°, 6° in 30 minutes. With animal E on January 24 the rectal temperature changed from 25.9° to 34.7° in 30 minutes. With animal F on January 24 a rise in body temperature from 28.8° to 36.1° occurred in 31 minutes, still another striking case of the rapid change which can occur in this relatively short time. Waking is, therefore, a much faster process than entering hibernation.

We have shown that the temperature of the animal in hibernation is constant at various points in the body from the mouth to the anus. Some of the early writers have set forth the idea that the marmot does not awake uniformly through the body, the anterior portion of the body regaining its normal functions much earlier than the posterior portion. If this is true, the animal should have considerable differences in temperature in different parts of its body during the waking process. In fact, it is quite logical to predict

TABLE 46—*Differences in rectal temperature of marmot No. 26 after faradic shock (February 10, 1937)*

Depth from anus	Rectal temperature at		
	5.35 p.m.	5.40 p.m.	5.42 p.m.
mm.	°C.	°C.	°C.
25	15.1	17.3	
63		21.2	
97		22.4	
120	22.4	24.1	24.6
143	23.3		

that there would be such differences with such a rapid transition, and a temperature difference actually does exist. Some data on this point are shown by an experiment on animal No. 26 on February 10, 1937, in table 46. The animal was awakened in this instance by a series of faradic shocks. The rectal temperature at 25 mm. insertion of the thermometer from the anus was 15.1°. At a depth of 120 mm. it was 22.4° and at 143 mm., 23.3°. The difference here between the temperatures at the two extreme depths is 8°. To further confirm this picture the mercury thermometer used was again withdrawn to the 25 mm. depth, and the temperature was found to be 17.3°, at this depth having warmed up 2° in 5 minutes. Temperatures at other depths were then recorded, which showed a regular increase in the temperature as the depth of the thermometer insertion increased. By this time the temperature at the 120 mm. depth had increased to 24.1° and two minutes later at the same depth it was 24.6°. Thus, marked differences in temperature are found at different depths in the body during waking.

In another series of measurements data were secured that not only demonstrate a temperature differential at different depths in the body at a given

time but furnish striking evidence that during the waking process the central part of the marmot's body approaches the warm-blooded temperature level more rapidly than does the posterior part. Simultaneous measurements of the rectal temperature of marmot No. 32 at depths of 25, 75, and 150 mm. from the anus were made on January 17, at an environmental temperature of 6° C. Previous to this date the marmot had been hibernating. Observations made every half hour between 3 and 6 p.m., during which time the respiration rate increased from 6 to 19 respirations per minute and the animal was obviously waking, showed temperatures in the rectum at the given depths from the anus as follows:

Time	25 mm.	75 mm.	150 mm.
3.00 p.m.	6.9	5.3	7.0
3.30 "	6.7	5.5	8.2
4.00 "	6.5	6.0	9.5
4.30 "	6.8	7.9	12.9
5.00 "	6.9	9.9	18.0
5.30 "	8.6	17.0	27.4
6.00 "	11.3	14.2	33.2

The same marmot, while hibernating on February 8 at an environmental temperature of 8° C., had a body temperature of 10° C. at all three depths from the anus. Eighty minutes later, during which time it was waking, the temperature at 150 mm. from the anus had risen to 24.2°, that at 75 mm. was 14.3°, but that at 25 mm. was still 10° C. Three hours after the initial measurements the temperatures at 150 and 75 mm. were both 36.1°, whereas that at 25 mm. was only 31.4° C.

It is unfortunate that we do not have more data on the body temperature gradient during waking, but as the animal wakes up it is impractical to have junctions in the mouth, for as the animal stirs it would be constantly changing the position and adjustment of any registering device. It is clear that as the animal comes out of hibernation it effects large changes in body temperature with surprising rapidity, during which time there is not a uniform body temperature, but marked differences in temperature at different depths in the body, the central portion being warmer than the peripheral tissue.

In the four illustrations shown in figure 10 the rectal temperature ceased to rise after it reached the normal warm-blooded level. As the rise was so rapid, it is perhaps surprising to find that the marmot has such a delicate regulation of its body temperature during waking. (See page 209 for further discussion of this point.)

RESPIRATION RATE DURING WAKING

Although the respiration rate is not a highly accurate physiological index of the other processes occurring in the body, since the volume of the respiration can vary greatly, it is obvious that the slow rates below one per minute, which have been shown to exist frequently in hibernation, cannot suffice for an animal in which the minimum rate when awake has been found to be 25 to 30. The respiration rate must necessarily increase as the animal awakes.

Unless the respiration rate is associated with other functions for compari-

son, only crude deductions can be made from changes which occur and these would have only slight value. The respiration rate during waking cannot be observed along with other measures, especially the heat production, unless the animal is in a well-illuminated glass chamber, or there is a glass window in the chamber. As the animal wakes up its activity will eventually over-shadow the respiratory movements and thus the measurements cannot be accurately observed. Hence data on respiration rate in waking do not abound in our observations.

However, in a few cases the respiration rate as the animal woke from hibernation has been observed. Animal No. 26 on January 30 was hibernating in the early morning, as it had been for several days previous. The respira-

TABLE 47—*Respiration rates of marmots during waking from hibernation*

Animal and date	Time	Rate per minute	Animal and date	Time	Rate per minute
1934	p.m.		1937	a.m.	
I			26		
March 14	2.15	14	January 30	7.35	0.4
	3.00	4		9.12	18
	4.30	0.8		9.54	67
	5.15	1		9.58	59
	7.20	0.5		10.02	66
	8.02	0.5		10.32	31
	8.38	0.5		10.51	24
	9.30	1		10.57	27
	10.12	1		10.59	22
	11.07	0.7		11.00	26
	11.19	0.8		11.05	19
				11.12	17
				11.18	13
	12.22	0.7		11.26	12
	12.32	4		11.28	11
	12.52	4			
	1.47	6			
	2.25	8			
	2.42	45			
	3.15	10			
	3.55	10			
	4.57	8			

¹ Animal handled just prior to this.

² Chamber warmed.

tion rate had varied from 0.3 to 1.0 per minute in the hibernating condition measured on the previous days. At 7.35 a.m. on January 30, the rate was 0.4 per minute. This animal, in addition to being handled to connect electrodes to the paws, was waked by putting its chamber in a warm bath. After it was handled, the respiration rate was observed to be 18 per minute at 9.12 a.m. The chamber was immersed in a warm bath at 9.30 a.m. and 24 minutes later the rate of 67 per minute was noted. This rate was maintained through 10.02 a.m., but from that time on the rate fell (see table 47), finally settling down to 11 per minute at 11.28 a.m., when the rectal temperature had reached the warm-blooded level of 37° C.

With animal I a similar picture was shown in an experiment on March 14,

1934. Placing the animal in a respiration chamber stimulated it somewhat, so that its respiration rate at 2.15 p.m. was 14 per minute, being irregular in its rate. By 3 p.m. the rate had decreased to 4 and during the evening it reached a level as low as 0.5. Starting at 10.15 p.m. the environmental temperature was raised to approximately 30° for the purpose of warming the animal. The respiration rate increased slowly and at 2.25 a.m. was only 8 per minute. Seventeen minutes later the fastest rate of 45 per minute was noted, but from this time on lower rates, averaging 9 per minute, were observed. The details are shown in table 47.

From these meager observations it is evident that in the initial steps during the waking process the respiration rate usually goes far above the ordinarily considered minimum level of 25 per minute when the marmot is awake and non-hibernating. It does not, however, at any time reach the very high level of 90 to 100 which has been noted with animals when awake. On the other hand, and we confess again the paucity of data, in at least two of the instances studied the respiration rates at the end of the experiment were actually considerably lower than what we give as the non-hibernating minimum. The respiration rate will be further discussed in connection with the correlation of the various functions with the heat production.

HEART RATE DURING WAKING

The rectal temperature and respiration rate have been noted to undergo profound and rapid changes during the waking period. In the discussion of hibernation the heart rate has been shown to be the best index of the heat production, so that the course of this function in the waking period will be of value in interpreting the corresponding changes which take place in the heat production. We know that the heart rate can descend to as low as 4 or 5 beats per minute with the animal in deep hibernation. The normal heart rate of the marmot when awake we have found to be 75 at its minimum and up to 200 in one instance when it was disturbed. The possibility of measuring the heart rate as the animal comes out of hibernation is affected by some of the drawbacks which we have cited in the case of the respiration rate, namely, that as the animal becomes active the muscular activity will prevent observation of the heart rate. The apparatus used for determining the heart rate was dependent on the electrical impulse set up by the action of the heart, but other currents caused by muscular activity also affected it. As the animal frequently became active, it was impossible to discern its heart rate impulses. However, some measurements were made before the animal became active and in other instances the animal did not become excessively active until fully awake. Animal No. 26 hibernated for several days, and the experiments are reported in the section on hibernation. Heart rates were not measured. On January 30, when we were intentionally waking the animal by warming it, the animal was first removed from the chamber and the electrodes were attached in order to study the course of the heart rate during the waking process by warming. This experiment is compli-

cated by the fact that the stimulus of handling the animal would of itself increase various functions. The heart rate measured from 8.50 a.m. to 9.10 a.m., after the animal had been returned to the cold chamber, was found to be 45 to 50 per minute. Gradual rises in the heart rate then occurred with rates reaching 67 per minute. At 9.24 a.m. two different counts established that the level had risen to 80, although counts one and two minutes before were as low as 50. From 9.24 a.m. on, the rate was 70 or above. Shortly after this the chamber was placed in a warm bath and at 9.31 a.m. the heart rate reached 100. Rates of about 110 were maintained from 9.36 a.m. to 9.46 a.m., when 120 beats per minute were found. By 9.53 a.m. the rate had increased to 158 and at 10.20 a.m. to 200. This is about as fast as it is possible to count by visual observation. For the next hour the rate remained in the vicinity of 200 or faster.

Animal M on October 9, hibernating, had a heart rate of 10 per minute at 11.37 a.m. At 3.15 p.m. the measure was 28 per minute. At 4.30 p.m. the rate was 56 and at 5.08 p.m. it had reached 100. No further observations were made, so that the maximum rate on this day is not known. On October 1, starting from an initial level of 11 to 12 beats per minute at 11.30 a.m. animal M increased its heart rate to 91 per minute at 2.30 p.m. During the next hour the rate rose steadily to 170 and one hour later had reached the 200 mark.

It will be noted that these heart rates reach a level higher than any found when the animal is normally awake, even higher than in an excited state. The rate of increase is rapid, and the high rate of 200 or over per minute which it attains is maintained for a considerable length of time. These tremendous changes in rate can occur within a period of an hour or two.

The study of the rectal temperature, respiration rate, and heart rate has brought to light the fact that in waking profound changes in these functions occur, and these changes take place in a relatively short time. A rise in the rectal temperature in such a short time to 37° from an initial level of 5° to 10°, while the marmot is maintained at an environmental temperature of about 10°, must be accompanied by an appreciably high heat production. Likewise the increase of the heart rate from a potential minimum of 5 to more than 200 per minute, a level appreciably higher than that usually noted even when the animal is excited when normally awake, would indicate that some tremendous change in the metabolic processes occurs during the waking.

THE COURSE OF THE METABOLISM DURING THE WAKING PERIOD

Since in our research emphasis was laid chiefly upon the gaseous metabolism from which the energy production can be computed, observations were made with the idea of contributing information regarding this important activity. Many of the animals, when they gave signs of waking, were placed inside a respiration chamber and their metabolism was measured. Frequently the waking occurred without external warming or stimulation, in

other words, was due to one of the many causes of normal waking. Other animals were definitely warmed by changing the environmental temperature, and in one instance the animal was given electrical shocks. Obviously the most perfect study of the marmot's metabolism in the waking period would be represented by observations made upon the animal when in the respiration chamber with the electrodes adjusted for records of the heart rate, with the thermo-junction in the rectum for rectal temperature measurements, and with provision for visual counting of the respiration rate. At the start of such experiments the marmot should be in deep hibernation, that is, with a respiration rate of approximately 1 per minute or less, with a heart rate of approximately 5 per minute, with a metabolism approximately 28 or 30 calories per $10w^{\frac{1}{4}}$, and with a low rectal temperature, usually 12° or below. If under these conditions the animal could be observed and then awakened, we could then study the entire process of passing from complete torpor to complete activity. This was rarely possible for us in its entirety. Occasionally reasonably complete observations were obtained, but for the most part we must depend upon incomplete data during the awakening process. Fortunately with our large population for study, a composite picture is built upon the observations on a number of animals and we feel we have sufficient data to establish the true physiological processes of the marmot during waking.

THE AWAKENING OF ANIMAL A

One of our earliest experiments, with animal A on December 21, 1931 (see table 48), has shown in a striking manner the physiology of transition, although of short duration, and although it is incomplete in that the true hibernating state is not here recorded, the protocols show that the animal when placed in the chamber at 9.45 a.m. was in a stupor. The eyes were closed but the animal did give a grunt.

Unfortunately no measures of the metabolism at the hibernating level were made at this time. The value commonly ascribed to deep hibernation is 27 calories, but as the animal gave a grunt, it was probably somewhat above this level. There were then seven periods of measurement from 10.22 a.m. to 4.19 p.m., the first period commencing after the animal had been in the chamber about 37 minutes. This first period, which lasted nearly one hour, showed the extraordinary rate of heat production of 2600 calories per $10w^{\frac{1}{4}}$, or a metabolism nearly 100-fold that in deep hibernation. The subsequent course of the metabolism is, however, of interest for in successive periods of approximately 50 minutes each, following the first period with its 2600-calorie rate, the values were 1790 and 1050, and thereafter remained essentially constant at about 830 calories for four periods. The rectal temperature at the conclusion of the experiment was practically that of the normal animal, 36.1° C. The level at which the metabolism settled, 830 calories, is notably higher than the value of 410 calories, the basal value established with an environmental temperature of 28° C. Here the animal is in an environment of about 11° and hence we have to deal with a very strik-

ing chemical regulation due to the lower temperature. The animal was definitely awake as indicated by the rectal temperature at the end of the experiment, and it was observed to be awake from December 22 to 25.

On January 2 the same animal, under similar conditions of experimenting (see table 48), during awakening showed inside of 2 hours in four periods beginning at 10.42 a.m. with, however, varying lengths of the periods, 2190 calories per $10w^{\frac{3}{4}}$ per 24 hours in the first 40 minutes, 1760 calories for 35 minutes, and 1340 and 1130 calories, in two periods of about 20 minutes each. The environmental temperature was approximately 11° and the rectal temperature after the experiment ended was 36.2° C. We have no records as to the condition of the animal as it was admitted to the chamber, but the very fact that it had a heat production of 2190 calories, that is, five times the normal basal rate, shows that the animal was recovering from the hibernation stage and giving essentially the same picture as on December 21, save that the experiment was not continued long enough for the animal to settle down to a level. If the same temperature reaction were to obtain, we would have expected the animal to settle down to about 900 calories in the course of time. Neither of these experiments began when the heat production of the animal even approximated that of hibernation, which shows that they were well on the way to waking when the first period began. It is, however, worthy of note that this animal, completely immobile and in stupor when placed in the chamber on December 21, could in three-quarters of an hour establish a heat production per $10w^{\frac{3}{4}}$ of such enormous rate, five to six times the normal non-hibernating heat production.

THE AWAKENING OF ANIMAL B

On January 4, 1932, animal B appeared to be in a stupor but had its eyes open. Particular care was taken not to handle the animal excessively prior to the metabolism test. A series of eight periods was made, each of approximately 45 minutes' duration. (See table 48.) At the end of the experiment the rectal temperature was 36.1° C. The environmental temperature was essentially 12° throughout the entire series. Here again the first records show a high metabolism of 1630 calories, thus proving the animal must have already entered well into the recovery stage. The heat production settled down in the succeeding periods to 1310, 1130, 800, and for the next four periods the values were between 640 and 780 calories, averaging not far from 700 calories. This average is a little lower than the 830 calories found with animal A on December 21, both of which probably represent the normal chemical reaction to environmental temperature.

Animal B was measured again on February 23, 1932. (See table 48.) Here we have the first indication of an experiment where the animal is measured at essentially the deep-hibernating level, although slightly higher, and during the actual experimental sojourn inside the chamber wakes up with the characteristic intense metabolism. The animal had been living at an environmental temperature of 9.5° , and the rectal temperature measured at

this time was 10.8° C. The marmot was then placed in a chamber at an environmental temperature of 27.5°, and nine experimental periods were made. The animal was asleep when the rectal temperature was measured

TABLE 48—*Metabolism of marmots A, B, and D when waking from hibernation*

Marmot, date, days fasting, and body weight	Length of period	Temperature		Heat pro- duction per 10w ^{2/3} per 24 hrs.	Marmot, date, days fasting, and body weight	Length of period	Temperature		Heat pro- duction per 10w ^{2/3} per 24 hrs.
		En- viron- mental	Rectal ¹				°C.	°C.	
1931-32	min.	°C.	°C.	cal.	1932-33	min.	°C.	°C.	cal.
Marmot A					Marmot D				
Dec. 21	57	11.9		2600	Dec. 19	179	6.5	5.4	2130
24 days	54			1790	7 days	50			1430
1.4 kg.	49			1050	1.1 kg.	65			1030
	49			820		83	8.5	35.2	1240
	50			800					
	50			850	Jan. 4	60	20.6	17.8	110
	48	10.9	36.1	850	23 days	60		16.4	120
					1.0 kg.	60		20.1	950
Jan. 2	40	10.4		2190		60		33.1	2320
36 days	35			1760		60		35.3	680
1.3 kg.	22			1340		60		35.7	650
	20	11.9	36.2	1130					
Marmot B					Jan. 31	60	15.3	9.0	26
Jan. 4	33	13.0			50 days	60		13.5	18
33 days	45				1.0 kg.	60		16.7	680
1.8 kg.	44					60		29.3	1790
	44					60		36.1	870
	48					60		36.3	560
	49					45	20.8		530
	52								340
	50	12.3	36.1	780	Feb. 14	76		5.4	240
					64 days	111		5.8	260
Feb. 23	37	27.8	10.8	130	1.0 kg.	59			450
46 days	39			80		77			740
1.4 kg.	43			60		60		8.6	2210
	48			2130		26			2170
	48			3930		21			1930
	41			810		18		14.3	2020
	67			630		18		17.5	1990
	62			480		9		20.9	1790
	37	26.5	36.6	500	Feb. 23	23			1590
					73 days	46			1080
					0.9 kg.	53			1400
						55		33.7	750
						46		33.2	660
						44			790
						45			810
						45			940
						26		35.2	800

¹ 100 mm. from anus.

² 150 mm. from anus.

and did not open its eyes, but was able to move and make a noise. The animal was placed in the chamber at 9.41 a.m. and the first periods of measurement indicated it was hibernating, that is, values of 130, 80 and 60 calories were found. In the next period of 48 minutes' duration this animal showed

a heat production averaging 2130 calories, followed by a 48-minute period when the heat production was 3930 calories. As the lowest value measured on this day was 60 calories, a 65-fold increase in metabolism occurred in about an hour. This emphasizes the intensity and rapidity of the metabolic rate. This extraordinarily high value was followed by four periods giving 810, 630, 480, and 500 calories. It is concluded that in the last two periods the metabolism had reached a plateau which is but little above the normally ascribed level for basal. It will be noted that the animal had been maintained for several hours at 27°, that is, about in the zone of thermic neutrality. The rectal temperature was 36.6° at the end of the experiment, which is evidence that the animal was awake. The next morning, February 24, the animal was still awake. The value of 3930 calories is absolutely the highest measured value that we have on any of our animals. Referred to the 27-calorie average of deep hibernation, it represents nearly a 150-fold increase in metabolism possible during waking.

THE AWAKENING OF ANIMAL D

The first waking experiment with this animal was on December 19, 1932 (see table 48), when it had been living at a very low environment of 3.7°, and during the experiment the temperature remained under 9°. The animal was considered to be hibernating at 9 a.m., and the rectal temperature of 5.4° is evidence in this direction. The gaseous measurements began at 10 a.m., but the extremely long period obscures the probable peak of the transition stage. The average heat production measured was 2130 calories. In the successive periods the metabolism remained at approximately 1200 calories, the rectal temperature being 35.2° at the end of the experiment. The 1200 calories again represents, without doubt, the influence of the extremely cold environmental temperature. The next morning the animal seemed to be going into hibernation. This experiment, although it occurs after a relatively short period of fasting and after a short period of hibernation, has no striking feature to distinguish it from other waking experiments.

On January 4, a second waking experiment was made with animal D. (See table 48.) It had been hibernating several days up to January 1 and was awake and dopey on January 2 and 3. It was placed in the chamber on January 4, with a thermo-junction inserted in its rectum, thus permitting continuous observations of the rectal temperature. During the first hour this was high (17.8°) and slightly lower in the second hour (averaging 16.4°) and the heat production was still remarkably low, 110 and 120 calories, respectively, for the two hours. There was then the characteristic rise, reaching a peak of 2320 calories and settling off again to the low value of 650 calories which represents approximately the heat production of an animal with a temperature a little below thermic neutrality.

On January 31 with this animal (see table 48) we had the good fortune to obtain the first two periods in the hibernating state with a heat production of 26 and 18 calories, respectively, for the first two hours. The simu-

taneously measured rectal temperatures averaged 9.0° during the first period and rose throughout the day to 36.3° C. In successive periods the heat production rose but did not go above 1800 calories, settling down finally to about 500 calories, and in the last period it was extraordinarily low (340 calories) in view of the environmental temperature of 20°, *i. e.*, much below thermic neutrality.

A perfect coordination of the heat production and environmental temperature with an animal just awakened is not to be expected. In general, however, it is seen that when the environmental temperature is in the neighborhood of 28°, the heat production levels off at a lower level than with colder room temperatures. Clearly it is shown that when the environmental temperature is very low, the animal never has a low heat production after waking, and it is usually very high.

On the morning of February 1, although the animal had finished the experiment on the day before with a temperature of 36.3°, the rectal temperature was down to 8° with the environmental temperature in the garage about 6° C.

On February 14, the marmot had been living at a low temperature of 3.5° and was measured presumably at this low temperature, but the records do not show precisely. (See table 48.) The rectal temperature is available beginning at 5.4° and the heat production, although high at the start (240 and 260 calories) when referred to the hibernating level, is very low when referred to the awake level at a cold environment, but mounts regularly to a maximum of 2210 calories and maintains nearly this level throughout the rest of the experiment. Here again this high level is determined without doubt by the extremely low environmental temperature of about 4.5°. The rectal temperature apparently did not go above 20.9°, but there is no record of whether the junction was inserted to its normal 150 mm. depth. Failure to do so might account for a low temperature.

On February 23rd at an environmental temperature of 6° or thereabouts evidently the marmot had already commenced to wake up, for the heat production was 1590 calories. (See table 48.) The metabolism fell in successive periods, settling at about 800 or 900 calories—an extremely low heat production for the cold environmental temperature. This extraordinarily low heat production in spite of a cold temperature suggests loss of vitality of the animal and that it is approaching a moribund condition. The animal died four days later.

THE AWAKENING OF ANIMAL E

This animal (see table 49) was studied on January 24, having previously lived at a temperature of 10.5°. The chamber was externally warmed during the day from 14.6° to 23.2° C. The first rectal temperature was 13.1° C., accompanied by a very low heat production, a true hibernating level, 36 calories per 10w¹. This low value is seemingly at variance with the relatively high rectal temperature but is further proof of the disassocia-

tion of the heat production and rectal temperature of these animals in the hibernating state. The second period of 135 minutes gave a low value of 110 calories with an average rectal temperature during the measurement of 16.8°C . In the next period the temperature averaged 26.2° , and the heat production 1600 calories. This again shows clearly the rapid rise with com-

TABLE 49—*Metabolism of marmots E, F, and G when waking from hibernation*

Marmot, date, days fasting, and body weight	Length of period	Temperature		Heat pro- duction per $10w^{2/3}$ per 24 hrs.	Marmot, date, days fasting, and body weight	Length of period	Temperature		Heat pro- duction per $10w^{2/3}$ per 24 hrs.	
		Envi- ron- mental	Rectal ¹				Envi- ron- mental	Rectal ¹		
<i>1933</i>										
Marmot E	min.	°C.	°C.	cal.	Marmot F	min.	°C.	°C.	cal.	
Jan. 24	135	14.6	13.1	36	Jan. 9	60	19.8	11.4	130	
43 days	135		16.8	110	28 days	60		14.9	290	
1.9 kg.	135	23.2	26.2	1600	1.3 kg.	60		21.3	2470	
						60		33.5	1240	
Feb. 20	35		6.0	600		60		36.2	590	
70 days	33		6.6	820		60		36.2	700	
1.8 kg.	24		7.0	720						
	28		7.6	770	Marmot G					
	32		8.2	1660	Feb. 16	48	3.7	4.0	70	
	29		9.1	2960	66 days	50			2290	
	25		10.1	2720	0.6 kg.	68			1720	
	31		12.1	2330		61			1720	
	37		16.6	2190		28		4.6	35.0	1480
	39		35.7	1800						
	40			1570						
	40			1320						
	21			1080						
March 16	71		5.3	7						
94 days	65		5.3	13						
1.7 kg.	54	27.3	8.1	20						
	53	25.1	10.8	46						
	62	28.1	13.7	36						
	55	29.8	16.0	50						
	36	29.2	19.2	2680						
	25	28.1		2710						
	27	27.5		2050						
	35	27.9	38.1	1420						
	37	28.1		610						
	44	27.4		570						
	45	26.6	39.6	590						
	23	25.9	39.7	590						
March 21	60	5.2	5.4	170						
99 days	60	27.1	10.9	60						
1.8 kg.	60	40.5	23.5	1460						
	60	36.5	35.5	640						
	60	31.3	40.7	700						
	60	32.5	42.1	490						

¹ 150 mm. from anus.

ing out of the hibernating stage. The animal was reported to be hibernating again on January 25.

Prior to an experiment on February 20 (see table 49), marmot E had been kept at the low temperature of 4.8° . The rectal temperature was taken throughout the entire day until it reached normal, and the animal had dis-

lodged the instrument. Although the animal had probably been hibernating for several days, the first heat production measures were high, 600 to 800 calories, for the first four periods. This level was then followed by the rapid ascent to a level of 2960 calories and subsequently by the usual descent, the metabolism settling at the end of the day at a level of about 1100 calories, under the influence of the extremely low environmental temperature. The incentive or cause to wake is probably due to the fact that the animal was placed in a very small respiration chamber. Further note is made that the chamber was too small for the animal to be comfortable when awake.

Animal E had been hibernating for a week when measured again on March 16. (See table 49.) The first two periods were made with the chamber temperature about 5°, which was the previous environmental temperature. The heat production was extraordinarily low, 7 calories for the first period and 13 calories for the next. The precise level of these values, however, must be considered to be doubtful since only extremely small amounts of carbon dioxide and oxygen were involved. For example, in the first period the oxygen consumption is only 0.16 c.c. per minute. Nevertheless the two periods agree reasonably well, showing an extraordinarily low level, a level so low that we do not care to consider it a verified minimum for the animal. The average of these extraordinarily low values and the values in the next four periods is not far from 27 calories, which is the average true hibernating level. The rectal temperature change from 5° to 16° is without any significant alteration in the heat production, although the arithmetical increase is from 7 to 50 calories. This is not commensurate with the rise in rectal temperature from 5° to 16°, again emphasizing the absence of correlation between heat production and rectal temperature at these low levels. In the next period there is a tremendous rise to 2680 calories, over a 50-fold increase, this being maintained for over an hour. Meanwhile the animal is at an optimum environmental temperature of 29°. The rectal temperature rises to a maximum of 39.7°, which is definitely a pyrexia for this animal, and yet the heat production of this animal during the last periods is approximately 600 calories. Under these conditions, therefore, it would appear as if the pyrexia of 2° or 3° did not significantly affect the heat production. The animal was again definitely hibernating on the morning of the 17th.

The last experiment with this animal in which this transition was involved was March 21 (see table 49), after 99 days of fasting and at a weight of 1.8 kg. The animal had been maintained at a temperature of 5°, was then measured first at a low temperature of about 5° (with a rectal temperature of 5.4°), and then the chamber was warmed and indeed warmed to over 40°. During the day the rectal temperature rapidly rose. On the other hand, the metabolism showed only the one pronounced increase during the third period to a maximum of 1460 calories and then settled down to approximately 500 calories with, however, a tremendously high rectal temperature of 42.1° C. This pyrexia, to be sure, did not strikingly affect the metabolism, but the animal died about 4.31 p.m., an hour after the last period was completed.

Throughout the entire research we had been searching to see if during the animal's waking up processes with tremendous heat development there would be any evidence of the animal's warming itself not simply to its normal body temperature but to a certain extent over-shooting, whereby it would be definitely in a febrile state. This was hinted at in the experiment of March 16 with this animal, because the actual rectal temperature went to 39.7° whereas the environmental temperature never went above 30° . On March 21, however, we have the environment very high, as the chamber temperature actually went to 40° . The resulting pyrexia was due, without doubt, not to the heat production itself, but to the environmental temperature. Furthermore the actual heat production for the various periods does not show any extraordinarily high values other than in the third period, which is one of the lowest observed in a waking experiment.

THE AWAKENING OF ANIMAL F

But one experiment (see table 49) during the transition period was obtained with animal F, on January 9, 1933. Prior to this date the animal had been living at about 6.5° for some time. It was hibernating on January 5 and 6 and drowsy after that time. The stimulus for waking was the intentional increase of the environmental temperature. The first hour gave a metabolism of 130 calories, showing the animal was not in the deepest hibernation and yet stuporous and well under its influence. The next hour the heat production rose to 290 calories, followed by the characteristic explosive rise to 2470 calories. During this time the rectal temperature showed the characteristic rise, attaining the normal level, however, only at the end of the fourth period. The heat production settled down to approximately 650 calories, which is not far from what is expected to be normal for an animal living in an environment of approximately 24° C.

THE AWAKENING OF ANIMAL G

But one waking experiment (see table 49) was made with animal G. Starting at the low rectal temperature of 4.0° C. the animal had reached 35.0° C. at the end of the experiment. The first period showed essentially a hibernating level of 70 calories and was followed by the explosion to 2290 calories, a level maintained for almost an hour. During the next two hours the heat production was about 1700 calories, followed by a period when it was 1480 calories, probably a result of the low environmental temperature, but the experiment was not continued far enough to know whether this last measurement would be the level at which the metabolism would settle or not. The animal had been hibernating for practically a week prior to this experiment.

THE AWAKENING OF ANIMAL I

An experiment (see table 50) with this animal on March 14, 1934, was characterized by being preceded by a well-established level in deep hiberna-

tion. It had been hibernating intermittently for a month, awake and going to sleep on the 9th, hibernating on the 10th, waking and going to sleep again on the 12th, and hibernating on the 13th. The respiration rate shows the characteristic low value for deep hibernation of 1 per minute or under. The first metabolism measurement was high, possibly due to the handling and transfer to a chamber, but the marmot did not wake. The metabolism then settled to approximately 42 or 43 calories, with a respiration rate somewhat under 1 per minute. The waking process was induced by an increase of the environmental temperature, but in this case the heat production rose very slowly and a number of intermediary steps are noted, 170,

TABLE 50—*Metabolism of marmots I and K when waking from hibernation*

Marmot, date, days fasting, and body weight	Length of period	Respiration rate per min.	Environmental temperature	Heat production per $10w^{2/4}$ per 24 hrs.
1934	min.		°C.	cal.
Marmot I March 14 58 days 1.2 kg.	40	7	9.0	290
	52	2		70
	46	0.8		60
	53	1		50
	84	0.7		40
	70	0.5		42
	108	0.5	9.1	43
	94	0.8	20.8	46
	108	4		44
	101	4		170
	40	6		270
	42	8		150
	38	45		2190
	38	9		1790
	38	9		1140
	25	9	23.8	1020
Marmot K April 10 87 days 1.0 kg.	24		19.7	2380
	35			1430
	38			830
	36			610
	55			640
	60			830
	39			570
	87		26.3	550
	81		8.8	820

270, and 150 calories, before the explosion to 2190 calories occurred. The rectal temperature unfortunately is not available. With this animal the waking period is accompanied by some measurable activity, which in part accounts for both the intensity of heat production and possibly the sustained value. The respiration shows an increasing rate which parallels reasonably well the intensity of the metabolism during the metabolic rise, but a rate of 9 at the end of the experiment is accompanied by a metabolism of 1020 calories, whereas the rate of 8 earlier in the experiment is accompanied by a metabolism of only 150 calories, again establishing the lack of sharp correlation between the respiration rate and metabolism. It is regrettable that the

respiratory volume per minute could not be measured. This animal shows clearly the hibernating level of metabolism and the respiration rate, with the explosive value followed by gradual decreases.

THE AWAKENING OF ANIMAL K

This animal had had a rectal temperature of 33° on the morning of the 9th of April, living at an environmental temperature of 16°. On the morning of April 10, 1934, the rectal temperature was not taken, as the animal appeared to be hibernating, even though the environmental temperature was approximately 20° C. It was desired to measure the animal in hibernation, but the stimulus due to handling awakened the animal, so that in the first period (see table 50) of measurement the heat production was very high, 2380 calories. The high value in the first period is followed by decreasing values, the animal's metabolism finally settling (at an environment of 26°) to about 600 calories. There was then a rapid cooling of the chamber for two hours followed by another measured period. The heat production had risen due to the chemical regulation to 820 calories. During the day a number of respiration rates were measured, varying from a minimum of 11 to a maximum of 83 per minute. In this experiment the animal was reasonably quiet throughout all the periods. The next morning, April 11, the rectal temperature was 37° C.

THE AWAKENING OF ANIMAL L

This animal had been living at 10° C. for over a month. The metabolism was measured on January 10, 1935, with the chamber temperature at about 12° for five periods, and although there was considerable variability ranging from 16 to 80 calories (see table 51), the general level was not far from that of the deeply hibernating animal. The environmental temperature was then rather rapidly raised for the purpose of waking the animal, but it was without significant effect upon the metabolism for three periods, or approximately two hours. Then this was followed by the characteristic explosion to 2760 calories with a subsequent gradual lowering, the metabolism settling to a rate averaging about 325 calories for the last three periods with an environmental temperature of about 28°. At the conclusion of the experiment the rectal temperature was 35.7° and the respiration rate 82 per minute. This experiment was characterized by an extraordinary degree of repose throughout all the periods, and graphic records indicate no muscular activity during the entire experiment. Hence the increased heat production is not ascribable to an increased muscular activity.

On March 29, 1935, measurements were made at an environmental temperature of about 15°, on the average. (See table 51.) The rectal temperature at the end of the experiment was 36.5°C. The animal was put in the chamber at 10.15 a.m. and the respiration rate at 10.19 a.m. was found to be 1 per minute, suggesting fairly deep hibernation. The first metabolism period did not begin until 11.06 a.m., at which time the heat production was

260 calories per $10w^{\frac{2}{3}}$. This was then followed almost immediately by the characteristic rapid rise to a maximum of 3550 calories and the metabolism

TABLE 51—*Metabolism of marmots L and M when waking from hibernation*

Marmot, date, days fasting, and body weight	Length of period	Temperature		Heat production per $10w^{\frac{2}{3}}$ per 24 hrs.
		Environmental	Rectal ¹	
1955	min.	°C.	°C.	cal.
Marmot L				
Jan. 10	24	13.0		80
57 days	52			44
1.6 kg.	56			16
	37			18
	61			33
	59	19.2		90
	42			40
	49			34
	27			2760
	19			1920
	32			1200
	38			660
	39			350
	59			280
	50	28.0	35.7	350
March 29	51	12.0		260
135 days	51			3110
1.2 kg.	15			3550
	24			3060
	35			1670
	32			1140
	29			1030
	30			1000
	36			850
	35			730
	25	16.6	36.5	710
Marmot M				
July 9	20	4.0		920
54 days	20			1000
1.4 kg.	20			1710
	30			2780
	45			3040
	30			2440
	30			1750
	30			2470
	31	6.1	36.4	2540
July 13	30	5.5		1260
58 days	30			3790
1.4 kg.	15	4.6	38.1	2900
July 17	20	7.1		2400
62 days	20			1940
1.4 kg.	20			1510
	20			1670
	20			1310
	20	9.3	33.7	1200

¹ 150 mm. from anus.

then tapered off in successive periods to a level of about 750 calories, at an environmental temperature of 16° C. This series of values would suggest

that the handling occurring when the animal was put in the chamber caused the awakening. Sixteen minutes after the experiment the respiration rate was 72 per minute. This experiment shows the tremendous increases in heat production possible for these marmots in a very short time. This animal produced but 260 calories at 11.06 a.m., but inside of an hour had a 10-fold increase, with a still further increase a few minutes later. The final heat production indicates that chemical regulation of the heat production is successfully combating the environmental cold at 16° C., as the animal is maintaining a rectal temperature at its normal non-hibernating level.

THE AWAKENING OF ANIMAL M

Animal M was unusual in that this was the only animal that hibernated in the summer. The animal was maintained in a refrigerator throughout the entire time at an environmental temperature of about 6° C. and was measured frequently at this temperature. Although we have definite evidence with regard to the hibernating metabolism of this animal (discussed under hibernation), in none of the experiments in the transitory waking phase were we able to obtain a preliminary hibernating level. Thus, on July 9, 1935, when the animal was put in the chamber, it was stated to be hibernating, and yet the measurement of the heat production gave 920 calories (see table 51) and this increased in the successive periods to 3040 calories. With considerable irregularity during the next two hours the animal maintained the high level, probably in large part determined by the fact that the environmental temperature was extremely low, 4° to 6° C. The rectal temperature was, however, up to normal, the animal having completely warmed itself prior to the end of the experiment.

On July 13 the marmot was asleep or drowsy at the start of the experiment. (See table 51.) Nevertheless the heat production was 1260 calories in the first period, and rose to a maximum of 3790 calories. In the third period it was 2900 calories, again partly caused by the very cold environment.

The high level which the animal attained may partly be explained by the fact that the environmental temperature was low, but this experiment was also complicated by the fact that the animal showed a high degree of activity in the last two periods.

On July 17 the animal was stated to be in hibernation, but stirred when moved from the refrigerator. The extraordinarily high value found in the first period (see table 51), 2400 calories, shows that the animal at this time was definitely in the process of awakening. The rectal temperature at the end, however, did not go as high as one would expect, 33.7° C. The transferring of this animal from its cage in the refrigerator to a metabolism chamber was the stimulus for the animal to waken on each of the days discussed.

THE AWAKENING OF ANIMAL NO. 26

This animal presents an unusually satisfactory picture of the study of the waking transition, since we have a definitely established hibernating level of

28 calories in the first periods on January 30, 1937. (See table 52.) This value agrees well with measurements in hibernation on January 27 and 29. The initial respiration rate on January 30 was 0.4 per minute. The heart rate could not be obtained in the hibernating condition. As it was desired to obtain this during waking, necessary attachments of electrodes were made. This required opening the chamber and resulted in the sacrifice of part of the heat measures. Half an hour after resealing the chamber the heart rate had increased to 80 per minute. The chamber was then immersed in a bath at 30° C. Thus, there were two stimuli for this animal to waken, handling and warming. The time required to attach electrodes and re-establish conditions for measurement consumed about one hour. When measurements were resumed, the heat production was well into the explosive heat production phase, being 1960 calories. Fifteen minutes later it had risen to 3060 calories. It remained at a high level for some time and gradually decreased, until finally it was not far from 1200 calories. This level of 1200 calories with a heart rate of 190 and a respiration rate of 13 or 14 per minute is somewhat difficult to explain, particularly since the animal in this stage was definitely drowsy. The environmental temperature was practically 31°, somewhat higher than the 28° level considered as the critical temperature, but as the rectal temperature did not go above 37.3°, it was hardly high enough to indicate a pyrexia.

The heart rate study with animal No. 26 shows that the marmot may have a heart rate of 200 per minute or over. Since it will be recalled that the rate in deep hibernation may be as low as 5 per minute, this corresponds to a 40-fold increase in the heart rate itself. Equally striking is the fact that the respiration rate in deep hibernation, commonly stated to be 1 per minute or under, is in this case 0.4 per minute and rises to 66, which is a 150-fold increase.

In this experiment where the heat production was 28 to 29 calories for an hour and had been demonstrated to be at this level on previous days, an increase of actually 100-fold occurred in the heat production, and this within 2 hours. Certainly none of the familiar factors governing heat production are capable of producing this overwhelming increase.

On February 4 (see table 52) this animal presented an extraordinary situation. At an environmental temperature of slightly below 2°, and with a rectal temperature of 5.6°, the heat production was maintained at approximately 350 calories for more than two hours, accompanied by a respiration rate of 4 per minute and a heart rate of 20 per minute. The heat production was as high as that with many animals measured in the basal, non-hibernating condition when they have rectal temperatures of 37°, heart rates of at least 75 per minute, and respiration rates possibly as low as 10, but more usually 25 to 30 per minute.

As has been pointed out previously (see page 75) this is a chemical heat regulation, possibly to protect the animal from freezing, for when the environmental temperature is raised, this protective mechanism is no longer needed,

and the heat production falls. Although with marmot 26 the heart and respiration rates were but a fraction of the levels which they would have had

TABLE 52—*Metabolism of marmot No. 26 when waking from hibernation*

Date, days fasting, and body weight	Length of period	Temperature		Per minute		Heat produc- tion per $10^w^{\frac{2}{3}}$ per 24 hrs.
		Environ- mental	Rectal ¹	Heart rate	Respiration rate	
1937	min.		°C.			cal.
Jan. 30	30	10.4	10.9		0.4	29
70 days 1.4 kg.	30		10.9			28
	15	13.8	12.5	111	19	1960
	15		22.6	159	66	3060
	15	27.1	26.9	180	56	2900
	15		29.2	208	31	2660
	15		33.6	208		2190
	15		35.4	204	17	1790
	15		36.1	196	15	1270
	15	31.9	37.3	188	12	1120
Feb. 4	20	1.8	5.5	20	4.0	340
75 days 1.4 kg.	20		5.6	21	4.0	340
	20			19	4.0	340
	30					350
	31		5.7	37	4.0	340
	29					290
	30					230
	50	9.9	6.1	10	1.5	150
	55		9.5	55	7.0	640
	20		10.6	88		1280
	20		11.1		35	1700
	20		12.2			1670
	20		15.0			2600
	20		19.8			2930
	20		20.2			2890
	20	12.2	26.7			2920
Feb. 10	30	10.1		5	0.8	31
81 days 1.4 kg.	30			6		32
	30			5	0.8	30
	30			6		34
	30			5		32
	30			5	1.0	34
	40					31
	40					28
	30			5		43
	224			5		28
	20			8		60
	11			36		390
	15			50		870
	15			67		1150
	15			83		1830
	15			97		1860
	15	12.1		113		2120
	15			120		2230

¹ 50 mm. from the anus.² Intermittent faradic shocks began during this period and continued throughout the experiment.

in the non-hibernating condition with this heat production, they both decreased when the heat production fell to 150 calories.

Raising the environmental temperature was eventually a stimulus to waken, for the animal went through the characteristic "burning-up" period of waking. The heart rate gradually increased and at the end of the tenth period had reached 143 beats per minute, but the movements of the animal, although insignificantly affecting the heat measures, prevented accurate observation of heart and respiration rates thereafter. It will be noted that both heart and respiration rates were well above the non-hibernating, basal level and if it had been possible to continue measurements, they would undoubtedly have been found to be as high as those noted in the previous experiment.

The fact that the heat production increased at a slower rate than frequently noted gave opportunity to measure the change in metabolism step by step. The peak level of the heat production, 2930 calories, was maintained for at least an hour. The heat production measurements were not continued beyond this point, as the animal was showing signs of activity which would be great enough to contribute a measurable amount to the heat production.

The rectal temperature was not measured as deep in the body as usual, due to the fact that the thermo-junction, when inserted on February 3, could be inserted only to a 50-mm. depth without disturbing the animal. As has been shown in the hibernation section, this will probably not influence the early temperatures reported, since in deep hibernation there is practically no gradient in the body. From our rectal temperature studies in waking, we realize that the temperature at this point will be slower in rising than at the deeper insertion. After the animal had had a heat production of almost 3000 calories for at least an hour, the temperature was 30.4°, although the average for the period was only 26.7°. One hour later the temperature at this spot had reached essentially the non-hibernating level of 36.2°C.

The experiment on February 10 (see table 52) started from the deep hibernating level of metabolism of 32 calories, with the heart rate characteristic of the hibernating condition, 5 or 6 per minute, and a respiration rate under 1 per minute. The extraordinary feature here was that the animal was awakened not by handling, not by raising the environmental temperature, but by administering a series of strong electric shocks through the electrodes attached to the front paws, normally used for counting the heart rate. Although intermittent shocks began during the 24-minute period with a heat production of 28 calories, there was no effect on the metabolism until the next period began. Likewise, the heart rate stayed at a low level up to this point. From here on the heart rate increased steadily, going to 120 per minute, and there was a gradual rise in the heat production from 60 to 2230 calories. The rectal temperature was imperfectly measured and at the end of the experiment, inserting a mercury thermometer to 150 mm., we found that the rectal temperature was 24.5° C.

This particular experiment gives one of the best pictures of ascending or increasing metabolism as the animal is waking. We have many experiments in which we have not observed it until the marmots have already waked and

are becoming adjusted to the environmental temperature. Here a clear picture of the rise is shown with the successive periods, 390, 870, 1150, 1830, 1860, 2120, and 2230 calories, accompanied by a strikingly uniform increase in the heart rate. Although no measures beyond this point were made, we have noted in other experiments that the body temperature reaches the normal non-hibernating level only after the heat production has started its descent from the peak. Thus, the general picture for this experiment is very similar to those obtained by other methods of waking.

THE COURSE OF THE METABOLISM DURING THE WARMING PERIOD AFTER NEMBUTAL

ANIMAL NO. 24

By the use of cold and nembutal, which is ordinarily a short-acting drug, we caused this animal to simulate a hibernating condition. It has been

TABLE 53—*Metabolism of marmot No. 24 during warming after cooling under nembutal*

Date, days fasting, and body weight	Length of period	Temperature		Per minute		Heat produc- tion per 10w ² per 24 hrs.
		Environ- mental	Rectal ¹	Heart rate	Respiration rate	
1937	min.	°C.	°C.			
Jan. 19	17	10.5	11.1	19	4	27
59 days	12	33.2	15.0	31	10	50
1.8 kg.	33	35.5	18.0	45	18	300
	15	35.8	22.6	86	24	570
	15	35.9	25.3	119	17	410
	17	36.4	27.5	132	21	490
	15	36.5	28.5	167	20	470
	15	36.2	29.7	159	26	430
	30	36.3	30.7	164	22	360
	30	36.6	32.9	176	17	360
	30	36.9	33.9	420
	30	37.2	34.8	182	33	440
	54	36.8	35.6	176	31	480

¹ 150 mm. from anus.

reported (see page 173) that the animal remained in the hibernating condition or at a low metabolic level for a period of 12 days, and throughout this time it had very uniform levels of its various functions. On January 19 (see table 53) the initial levels were practically at the minimum, with a heat production of 27 calories, a respiration rate of 4 per minute, and a heart rate of 19 per minute. After these levels were established, the chamber was placed in a water bath sufficiently warm to bring its temperature up to 36° C. The next period (in which the heat production was 50 calories) was commenced 25 minutes after the chamber was first immersed in the warm bath. At this time the chamber had not reached the maximum temperature, the average chamber temperature for this 12-minute period being 33°. The heat production doubled, as did the respiration rate, but the reaction of the heart rate was not so great, reaching only 31 per minute. A period following this, of 33 minutes' duration, showed a heat production of 300 calories, with

faster heart and respiration rates than noted in the previous period. In the next period, which started approximately 1 hour and 15 minutes after immersion of the chamber in the warm bath, the heat production was the highest, 570 calories, the period being of relatively short duration, 15 minutes.

Successive periods, approximately 15 minutes in length, showed heat values in every case under 500 calories, although the heart rate continued to rise and the respiration rate was maintained essentially at the level attained with the highest heat production. The rectal temperature rose steadily during the experiment, having the advantage of both a warm environment and an increased heat production, which, however, in this case exceeded 500 calories for only 15 minutes. A continuation of the heat measurements showed a level of approximately 400 calories or slightly higher. Not until $4\frac{1}{2}$ hours after the initial stimulation did the rectal temperature attain the level of 35.6° C . At this time both the heart rate and the respiration rate were still near the maximum levels noted during the experiment.

From the analysis of these data several important points are evident. In this warming process after nembutal the respiration rate does not reach the levels noted during waking from normal hibernation, but the heart rate closely approaches the maximum levels noted with normal animals. The rectal temperature, although it reaches approximately the non-hibernating level, aided by the environmental temperature, does not rise as rapidly with the marmot under nembutal as with the normally-hibernating animal. Perhaps the most striking point is that the heat production at no time exceeds 600 calories, whereas in normal waking there is an explosive heat production which practically always attains at least 1700 to 2000 calories. It is thus apparent that the animal is still under the influence of nembutal. When the heat measurements were discontinued some voluntary movements occurred, but the marmot was not in full possession of its normal faculties. An hour later, the animal was removed from the chamber and was found to be flaccid, exhibiting no muscular tone. It appeared to be inert, although it had previously shown signs of consciousness. It was placed in a cage in a warm room at approximately 28° C . The next morning the animal had partially recovered from its stupor. It stiffened its backbone when picked up and extended its front feet. It was able to support its weight on its front feet, but required some support for the posterior part of the body. The marmot did not react to sound and did not maintain the standing position for any length of time. By the middle of the afternoon, which was more than 24 hours after the last measure, the animal regained sufficient consciousness to be too active to obtain a basal metabolism measurement. From sometime previous to the administration of the drug to more than 24 hours after the marmot had been warmed to 37° , it had not urinated. This extraordinary picture of the effects of nembutal when the animal is cooled is very striking, for even after a period of almost two weeks the animal still exhibited evidence of the drug in its body. It is reported that this drug does not affect metabolism and is eliminated from the body by excretion.

The entire picture of the warming-up process with animal No. 24, which had been given nembutal, presents such a puzzling situation that it is greatly to be regretted that further evidence of this nature is not available. The animal had been under continuous observation for some time. The metabolic level had been approximately the lowest observed for hibernating marmots. The rectal temperature was nearly constant for 12 days and the environmental temperature in the refrigerator had been held approximately constant. The heart rate remained low, but not so low as has been observed in deep hibernation. The temperature of the environment was rapidly raised from 11° to 35° C. by placing the chamber containing the animal in a bath of warm water. This process automatically started the rise in rectal temperature which assumed a fairly rapid course, nearly as rapid per hour as under ordinary conditions. There was a striking increase in the heart rate, but after nembutal the time required to approach a maximum of 200 beats per minute is greater than when the marmot is coming out of hibernation normally. The astonishing observation, however, is that the heat production never exceeds 570 calories and for the most part it is nearer 400 calories, about the level for normally hibernating marmots at an environmental temperature of about 28° C. It is quite clear that this animal under nembutal has not a chemical regulation to combat the environmental temperature, but the rapid increase in the heart rate, unaccompanied by a correspondingly increased heat production, is an anomalous situation.

In our discussion of cold-blooded animals and many of the hibernating experiments, emphasis has been laid upon the fact that the heart rate is more closely correlated with the heat production than any other measurable factor. Indeed, the explanation for the low metabolism of cold-blooded animals has been based on their low blood supply. The animals do not have a higher heat production simply because they can not bring to the cells sufficient oxygen and metabolites. In this particular case, however, it would appear as if, judging from the rate of the heart beat, the circulation, provided the volume per minute corresponds at all to the heart beat, must have been very great and, therefore, the cells must have been supplied with an ample amount of oxygen and an ample amount of metabolites without, however, a correspondingly great increase in heat production. It is conceivable that the cells can not burn more than the amount of oxygen required for the normal metabolism, unless they receive a stimulus that is incidental to the chemical heat regulation. This being eliminated in the case of the drugged marmot, the metabolism can go only as high as the maximum cell temperature will permit it to go. As a matter of fact, the highest metabolism happens to be in the fourth period, a rather short period, and the errors of observation might easily have made the value of 570 not truly representative of the metabolism for that period. The general level of 400 calories for the rest of the day, however, is unmistakably factual. This experiment taken by itself is certainly to be interpreted as opposed to the alluring theory that if the blood brings to the cells oxygen and metabolites there will be a corresponding metabolism to make

use of this material. It is obvious that further experiments with this animal and indeed with other animals under nembutal and severe cold are necessary to clear up this very complex problem.

It is of interest to state that experiments in precisely this direction are, at the moment of writing, being carried out at the Nutrition Laboratory by one of us (R.C.L.) with other warm-blooded animals. A complicated picture is presented by this single experiment with this animal, under thus far wholly unexplored conditions, which include the factors of great cold, the reaction to chemical stimulation, the effect of nembutal itself, and the proneness for these animals under normal conditions to go into hibernation. Hence until further damaging evidence is forthcoming, the theory that metabolism is controlled in large part by blood supply will be retained as the, at present, most probable representation of the condition of affairs.

GENERAL DISCUSSION OF THE PHENOMENA OF THE WAKING OF THE HIBERNATING MARMOT

Respiration rate. Although many experiments were fortuitous and, therefore, often incomplete, a certain group of experiments were definitely planned and from the combined data we are able to formulate a fairly clear picture of the marmot as it wakes from hibernation. Although respiration rates were not noted in a large number of the experiments, there is evidence that the respiration rate did not surpass or even reach the maximum capable by the animal when awake. When we consider that the heat production during the waking process usually reaches at least 2000 calories per $10w^{\frac{1}{4}}$, five times the normal non-hibernating level, the usual maximum respiration rate during waking of only twice the usual non-hibernating average is surprising. The maximum noted for any period was 66 per minute. It also should be noted, however, that in the non-hibernating condition occasionally values as low as 10 per minute were found. Considering it from another angle we see that a tremendous change did occur. Thus with the experiment on animal I the rate in hibernation was 0.5 per minute and the maximum noted during the waking period with the marmot was 45 per minute. This was nearly a 100-fold increase, which is a greater increase than occurred in the heat production on this day. The maximum respiration rate noted always coincided with the maximum heat production and usually did not remain at the fast rate for the subsequent periods, even though the metabolism was frequently in the vicinity of 1000 calories or more. Since the respiration rate is not the only factor determining the volume of the lung ventilation, it is probable that as the animal becomes more fully awake the volume of respiration increases and by varying this it supplies the large volume of oxygen necessary for this rapid combustion and removes the large volume of carbon dioxide produced.

Rectal temperature. The waking process, however induced, once set in motion invariably results in the complete waking, with the explosive heat production, and is accompanied by a rise in rectal temperature to the non-

hibernating level. This rise in rectal temperature is always slow at the start, and as the process assumes more momentum the rectal temperature also ascends more sharply until it finally reaches approximately 36° or 37° C. It has been seen that the rectal temperature changes are always behind the changes in heat production and frequently do not attain maximum levels until after the heat production has descended from its peak level. Even in the experiments where the heat production of the animal is assisted by a warm environmental temperature this process (the body temperature rise) has a considerable lag behind the heat production. With the tremendous heat being generated, frequently for a period of two hours or more, it is conceivable that the rectal temperature might exceed the normal warm-blooded level. This has been found in only two instances where the environmental temperature was as high as or higher than 37°, in which case it is impossible for the animal to lose any heat to the environment by radiation. Thus, the marmot has some very delicate mechanism for regulating the balance between heat production and heat loss, so that the animal attains precisely this level on each awakening. The rise in body temperature during the waking process is not uniform at different depths in the rectal passage, and the temperature of the central part of the body rises more rapidly than that of the posterior part.

Heart rate. In hibernation we have seen that the function which has the closest correlation with the heat production is the heart rate. It will be important to note if this correlation holds during this rapid transition where the heat production reaches such an abnormal height. We find that in hibernation the level starts at 5 per minute, and during the waking period may go somewhat above 200 per minute. This increase would not be of the same magnitude as that of the respiration rate or the heat production, since they frequently have 100-fold increases, whereas a 50-fold increase would be the maximum for the heart rate. The maximum heart rate of slightly over 200 may be maintained for a period of more than an hour. The close correlation between heart rate and heat production observed in the hibernating condition is not so clearly demonstrated here, for the maximum heart rates do not occur with the maximum heat production, and the heart rate lags behind the heat production, especially at the maximum levels. The rate of 200 per minute is considerably higher than the usual maximum height with the marmot when awake. The general picture is, however, that there is a greater association between the heart rate and metabolism than with any of the other physiological factors.

Heat production. A most unusual feature of the waking period is the tremendous height to which the heat production rises and the remarkable rapidity with which the transition occurs (clearly shown by Dubois⁴ and Henriques).⁵ Because these phenomena are so unusual, it was at first deeply regretted that we did not have direct heat production measurements. How-

⁴ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, p. 67.

⁵ Henriques, V., Skand. Arch. f. Physiol., 1911, 25, p. 15.

ever, it is hard to conceive of a more complicated situation for directly measuring heat production (not heat loss). With many of the various forms of calorimeter it might be perfectly possible to measure the direct heat given off by the animal, both in the form of sensible heat, as Armsby calls it, or in the form of latent heat of water vapor. This is not heat production, this is heat loss. If heat loss were to be assumed heat production, certainly no animal would present any greater error than this animal coming out of hibernation, for during this period there is frequently a change in temperature of the entire body of 20° to 25° C. During the process of waking there is a very marked gradient which certainly may be considered an immeasurable gradient, and to attempt to compute the amount of heat stored by the marmot in the process of waking, from measurements of body weight and any of the at present available measures of body temperature, is simply inconceivable. With these considerations in mind we see that direct calorimetric measurements would not contribute intelligible values to this subject. Thus the indirect heat measurements based on the oxygen consumption were relied on in our study.

With at least two animals the rate of the heat production exceeded 3800 calories per $10w^{\frac{1}{2}}$ for a short time, about 20 to 30 minutes. Other animals were noted with a rate of 3000 calories, which was maintained for 30 minutes or more. The increase has frequently been noted to be 100 times the hibernating level observed a short time previous. Thus *inside of two hours* the metabolism may rise from 28 calories to 3000 calories, as noted with animal No. 26 on January 30. Practically all the marmots attain a level of 2000 calories, and this is five times the normal, non-hibernating, basal level. The maximum value of nearly 4000 calories is ten times the basal value. This maximum heat production far exceeds any value we have ever observed as a result of voluntary muscular activity. Although in certain cases it occurs with the marmot in a cold environment, the cold itself is not the primary cause, for we have noted animals at the critical temperature of 28° C. with a heat production of approximately 3000 calories during this waking period. Following the peak, the metabolism settles down to a uniform value. This value is usually largely dependent upon the environmental temperature. The nearer this environmental temperature is to the zone of thermic neutrality, the closer these values approach, in general, the non-hibernating basal value of 400 calories. It seems justifiable to assume that there is some special mechanism present in the marmot which is responsible for this unusual phenomenon. We have noted that the initial entrance into hibernation is a slow process, occurring by steps, but subsequent transitions into hibernation may occur in a relatively short time. However, there is no indication that the waking process is different whether it occurs soon after the marmot enters hibernation or the last time the marmot goes through the process in the spring. Our data do not show any marked difference, whether the animal is stimulated by handling, by warming, by electric shock, or wakes from an internal stimulus. With the animal under nembutal we found that although the heart

rate, the respiration rate, and the rectal temperature all practically simulated the levels noted with the normal, waking animal, the heat production did not have the tremendously high level of the "burning-up period" which is always present with the normal, waking marmot. We can only conclude that the nembutal was still having an influence on the animal's controlling mechanism, even two weeks after injection. This would suggest to the pharmacologist that the hibernating animal may be of special value in studying the delayed effect of certain drugs. Indeed, it may open a new field for the pharmacologist.

RESPIRATORY QUOTIENT DURING WAKING PERIODS

The process of waking from hibernation is characterized by a tremendous heat production, which is attained in a very short period of time and is frequently maintained much above the basal level for one, two, or three hours. The animals that exhibited this unusual phenomenon had usually been fasting for some time and the large losses in weight show that there had been severe drafts upon body tissue.

It is known from other fasting studies that practically the first fuel storage of the body to be depleted is the carbohydrate, that normally only a small portion of the energy is supplied by the combustion of protein, and that a fasting animal burns almost exclusively fat. This would argue that the only possible substances remaining in the body are fat and protein.

Not infrequently an animal will go through this waking cycle several times during the winter, each time the process being equally vigorous. We, therefore, became concerned as to what fuel is used to perform this waking process and turned our attention to the respiratory quotient for the answer to this question.

The old theory was that in hibernation the marmot is continuously converting fat into carbohydrate, which accounts for the very low respiratory quotient purportedly found, and as it wakes the first material burned is this carbohydrate. Consequently on the basis of this theory one would expect a tremendous change in the respiratory quotient between the hibernating phase and the waking phase. It is conceivable that the waking phase may be so rapid and so intense that the previously stored carbohydrate is quickly consumed and, therefore, when the marmot is clearly awake, it may be back at the fat quotient level. On this theory the respiratory quotient will start in hibernation as low as 0.6. During the waking period quotients might approach 1.00. One may conceive for the moment that the respiratory quotient has jumped up towards 1.00 shortly before the animal has completely reached its maximum rectal temperature and heat production, that the respiratory quotient may settle back to 0.7 and the marmot will go on burning fat because the previously formed carbohydrate has been burned up in this transition state. This theory is rather alluring because it ties in with the whole question as to the mechanism of the tremendous increase in metabolism occurring in the waking period. With an animal that can raise its

metabolism for a short time at least, 30 minutes or more, from a potential level of 20 or 30 calories per $10w^{\frac{3}{4}}$ in hibernation, to 3000 calories (a 100-fold increase) in a waking period, there is what one might call a teleological reason for this tremendous burning. In other words, the animal by nature feels the urge to do one of two things. The marmot is starting from a dormant state and has to combat an enemy, which has invaded its burrow, as soon as possible, or it is the end of winter and the marmot has to get up and forage for food. Consequently it does not want to waste any time in getting adjusted for combat or forage. When there is a liberal supply of carbohydrate in the body, it is probably selectively burned in muscular work and hence theoretically might contribute towards a rapid adjustment to a high level of metabolic activity. This whole conception is really very alluring, but whether it is true for the hibernating state or not must be considered here.

If the true combustion quotient is above that of fat in the waking period, we must consider what possible sources could be drawn upon to supply material resulting in such a combustion. There are three possibilities. First, the marmot has a glycogen reserve which it conserves, despite the prolonged fasts that it has endured. Such glycogen might possibly be stored in the liver, and during this unusual situation the animal makes this available and uses it to perform this intense combustion. If such glycogen is present and is used, one would expect the respiratory quotient at some phase in the transition to become, or at least approach, a value of 1.00. A second possibility is that for this combustion the animal forms carbohydrate from its protein.

A final theory would be that the animal actually burns protein itself, as this might be termed an emergency requirement and would simulate the condition found at the last stages of an animal dying from prolonged fasting. If the nitrogen metabolism could be measured during this period, it would be very significant in establishing whether this could occur or not. In this last case the respiratory quotient would not rise nearly so high as it would if the combustion were being carried on by carbohydrate material.

When the marmot is waking from hibernation, as well as when it is entering hibernation, conditions exist that frequently prevent the determination of the true combustion quotients occurring at the time. Some of the dangers pointed out in discussing the respiratory quotient of the marmot entering hibernation are present in the waking stage, for here again the respiratory system is in a dynamic state and never in a static condition. During waking there is a tremendous increase in the gaseous metabolism, a tremendous change in the carbon-dioxide output, and a corresponding change in the oxygen consumption. Hence in any waking period, long or short, it is doubtful whether the measures actually correspond to the carbon dioxide *produced* and the oxygen *absorbed*. This argument holds against all respiratory quotient determinations. That the measured carbon-dioxide output and measured oxygen consumption represent a real oxidation at the time of measurement is indisputable only when the animal is living at a constant metabolic level, and certainly when it is coming out of hibernation its metab-

olism is anything but constant. This does not significantly affect the accuracy of the heat determination, but it profoundly affects the accuracy of the respiratory quotient determination. As the body tissues become warm, less carbon dioxide can be held by them. With the possibility of liberating and blowing off carbon dioxide a variable or a series of variables is introduced here which makes it very uncertain whether or not the resulting respiratory quotient is true. Consequently, although the study of the respiratory quotient during this transitory phase is important and helpful in indicating a trend, nevertheless it never can be looked upon as representing a true picture of what is termed by Carpenter a "combustion quotient." The previous literature has shown great changes in the respiratory quotients. They have usually been interpreted as representing real physiological facts, with particular reference to the combustion of materials, carbohydrate or fat or both, that would correspond to this type of quotient.

One must never lose sight of the fact that these are *Transition Quotients* and that, although "apparent" and suggestive, they are anything but definitive. It is important to look at these quotients in the light of the earlier experiences in the literature. We have already seen that the fantastically low quotients during hibernation frequently appearing in the literature are false and that the quotient of the hibernating marmot is essentially that of fat combustion. We may, therefore, logically challenge many of the quotients during the transitory period of coming out of hibernation. There have frequently been reported quotients of 1.00, if not over, contributing to the conviction that during this stage there is a selective, and at times an exclusive combustion of carbohydrate. Fortunately during the later stages of coming out of hibernation the intensity of the metabolism is so great, the amounts of gases involved are so great, that we can discount to a large extent any of the factors that so profoundly affect the respiratory quotient measurements when the metabolism is at an almost immeasurably low level, where a change of a few tenths of a milligram in the carbon-dioxide elimination may alter greatly the respiratory quotient. With a high production of carbon dioxide obviously these small errors are wiped out. One must, therefore, recognize that the several quotients obtained during the early stages of transition, that is, when the metabolism is at the lowest level, are subject to the greatest errors. As the metabolism increases, most of the errors in determining the quotients should rapidly, if not completely, disappear.

At least in the first stages of waking the measured respiratory quotient will be erroneous, and when we consider that during the entering process a large volume of carbon dioxide was shown to be retained, which must be given off in the waking process, we realize that the quotients will tend to be too high, *i.e.*, above the true respiratory quotient. Compared with the entering process, the waking process takes place in a short time, causing the rectal temperature to rise rapidly, thus liberating the carbon dioxide retained in the entering phase relatively quickly. The carbon dioxide liberated rapidly by the marmot in waking might be expected to have a greater effect on the

quotient than the slower reverse process had on the quotient when the animal was entering hibernation. Since the total metabolism rises so fast and to such a high level, the liberated preformed carbon dioxide may be only an insignificant part of the total carbon dioxide produced. From tables 48 to 52, which show heat production and rectal temperature, it is apparent that frequently the greatest body temperature changes occur, as one would expect, during the period of highest heat production. Thus any liberation of retained carbon dioxide due to warming will be greatest at this time so that probably even these quotients will not be "combustion quotients."

It would be helpful to have some approximation of the volume of carbon dioxide that could be retained by the marmot as it enters hibernation. Considering the rectal temperature for marmots in deep hibernation as 10° and awake as 37° C., we can estimate from the solubility of carbon dioxide in water the volume of carbon dioxide that could be held by the body, as water constitutes about one-half of the body weight. At 10° the solubility of carbon dioxide in water is 1.19 c.c. carbon dioxide per 1 c.c. water and at 37° about 0.55 c.c. per 1 c.c. Therefore, each gram of water at 10° can retain 0.64 c.c. more carbon dioxide than it can at 37° C. For a marmot weighing 1.5 kg., 750 gm. would be water. Hence when the body temperature is cooled from 37° to 10°, 750 times 0.64, or 480 c.c. of carbon dioxide could theoretically be retained, in addition to any carbon dioxide already stored at 37°, and the same amount would be liberated when the process is reversed, that is, when the marmot is warmed from 10° to 37° C. It is realized that the pH of the blood will determine the actual volume of carbon dioxide that can be retained and any changes in the pH will affect the carbon-dioxide storage.⁶ But it is evident that an appreciable volume of carbon dioxide can be stored or retained at the cold temperatures, in addition to any already present at the 37° level.

A summary of our waking data on *apparent* respiratory quotients, that is, the quotients measured, based on carbon-dioxide elimination and oxygen absorbed, are presented in table 54. It is strikingly evident that in no case is a value even approaching 1.0 found, despite the fact that, due to the liberation of carbon dioxide, quotients will tend to be high. There are a sufficient number of experiments which were continued until a plateau was reached after the "burning-up" stage to show convincingly that the entire phase has been studied.

The highest quotient measured was 0.83, and in general the values of 0.80 would be the maximum. If, for the moment, these values are assumed to be true quotients, it is clear that the "burning-up" process is not exclusively a carbohydrate combustion and that less than half the metabolism, even at the maximum quotients, can be attributed to this material. The concept of a pure carbohydrate combustion in waking is, therefore, not supported by our data. Since the respiratory quotient of protein is 0.81 or 0.82, it would be possible to interpret the measured quotients as indicating a wholly protein

⁶ Rasmussen, A. T., Am. Journ. Physiol., 1916, 41, p. 162.

combustion, but as it is recognized that the quotients due to liberated carbon dioxide will tend to be high rather than low, protein is not the sole probable source of energy.

In the experiment on animal No. 26 (body weight, 1.4 kg.), on February 10, 1937 (see table 57), the actual volume of the oxygen absorbed in the 2-hour waking period was 2770 c.c. The corresponding carbon-dioxide elimination was 2182 c.c. The apparent respiratory quotients during this time were all higher than 0.70 (see table 55), but none of them exceeded 0.81. Assuming

TABLE 54—*Apparent respiratory quotients of marmots in waking periods, at various levels of heat production per 10w³*

Marmot and date	Below 50 cal. (Avg.)		50 to 100 cal. (Avg.)		100 to 1000 cal. (Avg.)		1000 to 2000 cal.		2000 to 3000 cal.		3000 to 4000 cal.		3000 to 2000 cal.		2000 to 1000 cal.		1000 to 100 cal.	
	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum
Marmot D Feb. 14, 1933 " 23, "			0.69				0.77	0.78			0.77	0.78	0.78	0.79	0.79	0.83		
Marmot E Feb. 20, 1933 March 16, "	0.67	0.78	.71	0.81	0.81	.75	.76						.74	.76	.71	.73	.73	.73
Marmot G Feb. 16, 1933			.71				.73	.73					.75	.75				
Marmot I March 14, 1934	.64	.73	.74	.74	.77								.74	.74	.73	.74		
Marmot K April 10, 1934													.73	.74	.70	.71		
Marmot L Jan. 10, 1935 March 29, "	.71	.67					.71	.71	0.72	0.73			.73	.74	.73	.75	.71	.76
Marmot M July 9, 1935 " 13, " " 17, "			.72	.73	.74	.76	.76	.76	.72	.72	.73	.74	.72	.72	.69	.70		
Marmot 26 Jan. 30, 1937 Feb. 4, " " 10, "			.69	.75	.77	.77	.79	.79	.73	.73	.74	.75	.68	.69				
	.70	.77	.79	.80	.81	.78	.79											

that the combustion was one of fat,⁷ i.e., a respiratory quotient of 0.70, we compute from the oxygen value that the carbon-dioxide production equivalent to this oxygen consumption would be 1939 c.c. The difference (243 c.c.) between this and the measured carbon-dioxide elimination represents the volume of carbon dioxide assumed as previously retained and subsequently liberated.

⁷ We speak of pure fat combustion, but we refer to fat plus a certain small moiety of protein.

Animal No. 25 (body weight, 2.3 kg.), on December 4, was drugged with nembutal and cooled. The measurements of the gaseous exchange were not commenced until the rectal temperature (at 150 mm. from anus) had descended to 19.6° C., but the measurements were continued from this point until the temperature reached 8° C. According to our calculations shown in table 57, the carbon dioxide stored during this time was 127 c.c.

It will be recalled that when the marmot is entering hibernation, the apparent respiratory quotient, even with a slow ventilation rate, often does not come up to the fat quotient for a number of hours after a plateau of metabolism has been reached. This would indicate that the absorption or retention of carbon dioxide goes on for an appreciable time before equilibrium is reached. Therefore, even though the rectal temperature and heat production do not decrease any further, it is possible for a continued carbon-dioxide storage to occur. Thus, considerable volumes of carbon dioxide are retained when the marmot is entering hibernation.

TABLE 55—*Apparent respiratory quotients of marmot No. 26 when waking from hibernation (February 10, 1937)*

Length of period min.	R.Q.	Heat production per $10w^{2/3}$ per 24 hrs. cal.
24	.70	128
20	.77	60
11	.79	390
15	.78	870
15	.81	1150
15	.80	1830
15	.79	1860
15	.79	2120
15	.77	2230

¹ Hibernating period.

In the experiment where animal No. 24, cooled under nembutal, was restored to the warm-blooded level by warming, we find very high respiratory quotients appearing for a few minutes. (See table 56.) In this case two striking things are noted. First, the quotient actually reaches a level slightly above 1.00 for a short time. Secondly, the heat production never reaches the value of 600 calories. Since there is no burning-up period, the necessity for a special combustion process is not present. In this study we have continuous measurements of the respiratory exchange for a range in rectal temperature from 15° to 36° C., nearly the entire range from hibernation to awake. A calculation (in a similar manner as has been made for animals Nos. 25 and 26) of the volume of carbon dioxide liberated in excess of that during fat combustion shows that 339 c.c. of carbon dioxide have been eliminated. The amounts of carbon dioxide assumed to be stored when the marmot enters hibernation and liberated when it awakens are, therefore, of such an order that they could be accounted for by this theory. The measured quotients above that of fat are, therefore, not due to extreme

demands, since in this experiment with no extreme demands the highest quotients were found.

An attempt to apportion the carbon-dioxide retention, on the one hand, and the discharge, on the other, to the body weights of the several marmots fails, for animal No. 26, with a body weight of 1.4 kg., according to calculations has liberated 243 c.c., whereas animal No. 25, with twice this body weight, on entering hibernation has retained only 127 c.c., but both of these

TABLE 56—*Apparent respiratory quotients of marmot No. 24 during warming after cooling under nembutal*
(January 19, 1937)

Length of period min.	Rectal temperature ¹ °C.	R.Q.	Heat production per $10w^{2/3}$ per 24 hrs. cal.
12	15.0	.81	50
33	18.0	1.04	300
15	22.6	.84	570
15	25.3	.95	410
17	27.5	.85	490
15	28.5	.84	470
15	29.7	.81	430
30	30.7	.81	360
30	32.9	.83	360
30	33.9	.82	420
30	34.8	.95	440
54	35.6	.79	480

¹ 150 mm. from anus.

TABLE 57—*Calculations of carbon dioxide liberated or stored by marmots under different conditions*

	Marmot 26 ¹ (Feb. 10, 1937)	Marmot 25 ² (Dec. 4, 1936)	Marmot 24 ³ (Jan. 19, 1937)
Total O ₂ absorbed, c.c.	2770	703	2389
Total CO ₂ eliminated, c.c.	2182	365	2011
Total O ₂ absorbed $\times 0.7$	41939	4492	41672
CO ₂ liberated, c.c.	243	127	339

¹ Waking from normal hibernation.

² Under nembutal and cooled.

³ During warming after cooling under nembutal.

⁴ This represents the carbon-dioxide production corresponding to the oxygen absorption during fat combustion.

⁵ Carbon dioxide stored, not liberated.

experiments are incomplete. Animal No. 24, with a body weight of 1.8 kg., when warmed after cooling under nembutal is characterized by not having any of the so-called "burning-up" process and during nearly the entire warming phase liberated 339 c.c. of carbon dioxide. It is clear that in the case of these three animals there are no direct mathematical relationships between the carbon dioxide calculated as having been retained when the marmot was entering hibernation and that found to have been subsequently discharged as it came out of hibernation. There is, however, sufficient evidence, espe-

cially when we take into consideration the fact that direct observations⁸ have shown an increase in the carbon-dioxide content of the blood in the hibernating animal over that of the animal in the non-hibernating state, to show that we are dealing with material amounts of carbon dioxide that may be retained and subsequently discharged, amounts that are fully sufficient to account for any of the apparent divergencies in the respiratory quotient from that which would be ascribed to fat.

Analyses of the bodies of hibernating marmots⁹ and of the blood of a hibernating squirrel and dormouse¹⁰ and marmots¹¹ in hibernation have never shown a body completely free from glycogen in hibernation. It is conceivable that in the time of duress, or at the time of waking, there may be a small proportion of glycogen actually involved in the combustion. This, however, would be so small that if significant amounts of carbon dioxide were available from the body tissues, this would always remain as the highly probable explanation of these deviations of the quotient.

It is thus evident, as concluded by Henriques a quarter of a century ago,¹² that the awakening process is not performed by an exclusive carbohydrate combustion. Although glycogen and protein are probably burned in small amounts during this period, we conclude that the chief fuel for the explosive combustion is fat.

⁸ Rasmussen, A. T., Am. Journ. Physiol., 1916, **41**, p. 162.

⁹ Weinland, E., and M. Riehl, Zeitschr. f. Biol., 1907, **49**, p. 37.

¹⁰ Dische, Z., Fleischmann, W., and E. Trevani, Arch. f. d. ges. Physiol., 1931, **227**, p. 235.

¹¹ Britton, S. W., Am. Journ. Physiol., 1930, **94**, p. 686.

¹² Henriques, V., Skand. Arch. f. Physiol., 1911, **25**, p. 15.

APPROACHES TO ARTIFICIAL HIBERNATION

NEMBUTAL AND CARBON-DIOXIDE NARCOSIS

As has already been discussed (see pages 129, 153, and 172), with the use of nembutal we have been able to make the marmot simulate many phases of normal hibernation. In order to study more clearly the hibernating mechanism, we have tried inducing artificial hibernation by following the method of Dubois¹, using carbon-dioxide narcosis and cold. The usual method was to place the animal in the chamber, and then to introduce the gaseous mixture. (See page 21.) When the animal was sufficiently anesthetized to permit handling, it was removed from the chamber, the rectal thermo-junction was inserted, and the heart rate electrodes were attached. The animal was then returned to the chamber and the inflow of the gaseous mixture resumed. By the maintenance of a ventilation rate of one liter per minute the respiratory exchange did not alter the oxygen and carbon-dioxide percentages of the gaseous mixture.

One of the gaseous mixtures used was 45 per cent carbon dioxide and 55 per cent room air. Since room air is approximately one-fifth oxygen, the composition of the mixture was 45 per cent carbon dioxide, 11 per cent oxygen, and 44 per cent nitrogen. In all cases this mixture produced a fall in the heart rate, the respiration rate, and the rectal temperature, resulting in the animal's becoming narcotized. When the carbon dioxide in the mixture was discontinued, invariably the animal began to regain consciousness. Usually a temporary cessation of the carbon dioxide in the mixture immediately caused the heart rate and the respiration rate both to increase sharply, as can be noted in figure 11, page 221, from the trends of the curves at 5.30 p.m. The rectal temperature was relatively slow in reacting to a change in atmospheric condition. However, the rectal temperature falls soon after the mixture is administered. On November 10, with the administration of 35 per cent carbon dioxide and 65 per cent room air, the rectal temperature of animal No. 20 fell from 36.5° to 33° C. in the first hour, and the heart rate went from 100 to 51 beats per minute. The respiration rate, initially 33 per minute, was accelerated to 83 per minute by the carbon dioxide in the inspired air, but by the end of the first hour had decreased to a level of 38. In the second hour the temperature decreased to about 30.2°, a drop of less than 3°, whereas the respiration rate went from 38 to 20 per minute and the heart rate from 51 to 35 per minute. An adjustment of the heart rate electrodes, prior to the end of the third hour, temporarily increased the heart rate to 50 beats per minute and the respirations to 42 per minute, but the rectal temperature continued to fall, reaching 27.6° C. In the fourth hour the levels of the various functions were, heart rate, 38.5 per minute; respiration rate, 19 per

¹ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896, p. 250.

minute; and rectal temperature, 25.8° C. The environmental temperature during this entire experiment was maintained between 9° and 13° C. At the end of the fifth hour in this gaseous mixture the animal had a rectal temperature of 24° C., a heart rate of 24 beats per minute, and a respiration rate of 15 per minute. The systematic decrease in rectal temperature is strikingly shown when expressed as a 3.5° fall in the first hour, 2.8° in the second hour, 2.6° in the third hour, 1.8° in the fourth hour, and 1.8° in the fifth hour.

The percentage of oxygen (11 to 13 per cent) in the 45 and 35 per cent mixtures being lower than that in room air, it became a question as to whether the anesthetization was produced by the high percentage of carbon dioxide or the low percentage of oxygen. To illuminate this point, animal No. 14 was first subjected to a mixture of 45 per cent carbon dioxide, 11 percent oxygen, and 44 per cent nitrogen. On the following day the carbon-dioxide content of the air was the same (45 per cent), but the remaining 55 per cent was pure oxygen. The heart rates, respiration rates, and rectal temperatures at intervals of one and one-half hours, two hours, three hours, and three and

TABLE 58—*Carbon-dioxide narcosis of marmot No. 14 with varying oxygen percentages*
(Environmental temperature, 12° C.)

Date	Gaseous mixture			1½ hours ¹			2 hours ¹			3 hours ¹			3½ hours ¹		
	Per cent of			Heart rate	Respiration rate	Rectal temperature	Heart rate	Respiration rate	Rectal temperature	Heart rate	Respiration rate	Rectal temperature	Heart rate	Respiration rate	Rectal temperature
	CO ₂	O ₂	N ₂			°C.			°C.			°C.			°C.
1936															
Oct. 26	45	11	44	...	28	28.5	63	25	28.8	45	17	26.7	44	16	26.5
Oct. 27	45	55	0	...	30	29.5	45	19	29.1	42	13	26.6	38	12	25.6

¹ After start of anesthetization.

one-half hours after the start of the anesthetization on each day are shown in table 58. From this table it is clearly seen that the low percentage of oxygen in the gaseous mixture is not the anesthetizing agent, for with the 11 per cent oxygen, the rectal temperature had dropped in 3½ hours from its normal level (approximately 37° C.) to 26.5° C., and with 55 per cent oxygen to 25.6° C. After the same period of time the heart rate was 44 and 38 per minute and the respiration rate 16 and 12 per minute.

With this profound lowering of body functions, the question naturally arises as to how long an animal can live in an atmosphere of 45 per cent carbon dioxide. This varies with different animals. On October 13, 1936, in a 45 per cent carbon-dioxide and 55 per cent room-air mixture, animal No. 16, showing a continually decreasing respiration rate (the only function measured in this particular case), was in good condition at the end of five hours but three hours later, in the same atmosphere, the animal was found dead.

Animal No. 14 on October 27, 1936, was maintained in an atmosphere of

45 per cent carbon dioxide and 55 per cent oxygen for 12 hours, except for a half-hour period at the end of the fourth hour, and for an hour at the end of 9 hours when the carbon-dioxide content was lowered to 10 per cent. After being at 45 per cent carbon dioxide for essentially 12 hours the animal ceased breathing. The heart rate began to fall rapidly to the lowest rate that had been observed on that day, and the rectal temperature continued to fall. The animal was taken out of the chamber and resuscitated by the use of oxygen and artificial respiration. It commenced breathing normally inside of 10 minutes, and during the first hour after resuscitation the respiration rate did not exceed 7 per minute, the heart rate reached a maximum of 50, but averaged about 30 per minute, and the rectal temperature rose less than half a degree. During this period the animal lay on its back, with only

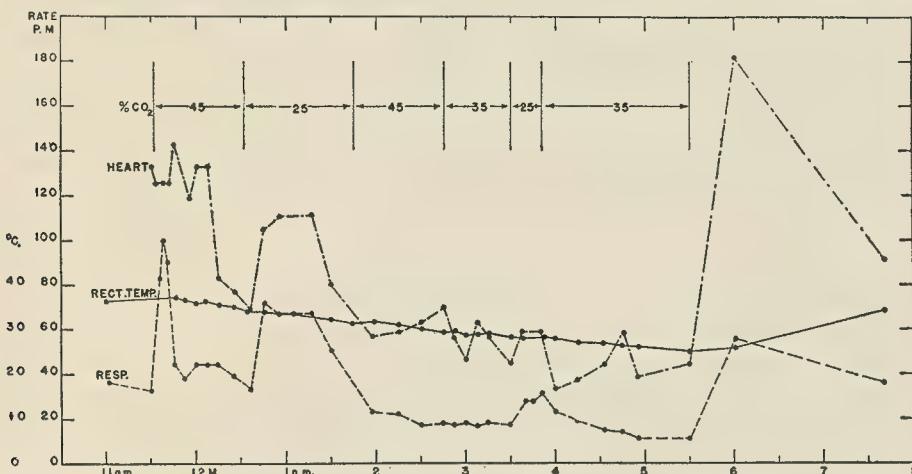


FIG. 11—CHANGES IN BODY FUNCTIONS DURING CARBON-DIOXIDE NARCOSIS WITH MARMOT 20, NOVEMBER 6, 1936, AT AN ENVIRONMENTAL TEMPERATURE OF 12° C.

Solid line represents rectal temperature; dash-and-dot line represents heart rate; broken line represents respiration rate. When not indicated, the chamber was ventilated with room air. When mixtures of room air and carbon dioxide were used, the percentages of carbon dioxide in the mixtures are indicated at the top of the figure.

respiratory movements. However, seven hours later, or eight hours after the animal had been breathing room air, the heart rate was 200 per minute, the respiration rate was 45 per minute, and the animal was fully conscious, having destroyed the rectal thermo-junction.

It was of particular interest to know if, after the animal became narcotized, the carbon-dioxide percentage could be lowered and subsequently dispensed with and yet have the animal maintain these low body functions. A graphic presentation, representative of our experimental data on this question, is given in figure 11. In this instance we were fortunate in being able to attach the heart rate electrodes and insert the rectal thermo-junction before anesthetizing the animal. This animal, No. 20, had been kept for some time at 8°, and on November 6 was placed in the refrigerator at 12° C. After the

stimulus of handling had passed, heart rates of 133 and 125 per minute were observed, the respiration rate in two counts was 37 and 33 per minute, and the rectal temperature was 36.5° C.

Initially the gaseous mixture containing 45 per cent carbon dioxide and 55 per cent air temporarily stimulated the heart and respiration rates. When the carbon-dioxide percentage was lowered to 25 per cent another transitory increase in these rates occurred. Return to room air at 5.30 p.m. caused a tremendous stimulation of both heart and respiration rates and, contrary to the general finding that the rectal temperature continued to decline when the carbon-dioxide content of the gaseous mixture was lowered, the rectal temperature here definitely increased.

This animal, at an environmental temperature of 13° on November 10, had a rectal temperature of 36.5°, a heart rate of 100 beats per minute, and a respiration rate of 33 per minute. After 48 hours of carbon-dioxide anesthesia (varying from 35 to 10 per cent carbon dioxide plus room air) the rectal temperature had decreased to 9.2° C., the heart rate to 16 per minute, and the respiration rate to 1 per minute. Room air was then introduced into the chamber. The heart rate, respiration rate, and rectal temperature increased slowly but steadily, and after the marmot had been 12 hours in room air the heart and respiration rates were 91 and 77 per minute, respectively, but the rectal temperature had reached only 18.1° C. Twenty per cent carbon dioxide at an environmental temperature of 5° C. quickly lowered the respiration and heart rates, but only after two and one-half hours did the rectal temperature begin to fall. During twelve hours at 20 per cent carbon dioxide the rectal temperature fell steadily to a level of 10.9° C., and the heart and respiration rates fell to 25 and 4.5 per minute. Eight hours after this, still in the gaseous mixture (20 per cent carbon dioxide), the animal was found dead.

The longest time an animal stayed in the narcotized state when the carbon dioxide was discontinued was 12 hours. There were no cases in which it was possible to lower the various functions with carbon dioxide and then maintain this condition with cold alone. The longest time during which we were able to maintain successfully the animals under carbon-dioxide anesthesia was about 80 hours in the case of animal 20.

From a number of experiments, not here presented, the evidence is that the carbon-dioxide content of the anesthetizing mixture may be gradually lowered (5 per cent every hour or two) to a minimum of 20 per cent and still maintain the animal in the unconscious state, and unless at least this amount of carbon dioxide is continually present complete narcosis cannot be maintained.

Under carbon-dioxide narcosis and cold (about 8° C.) the rectal temperature was lowered to 10° C. on several occasions, accompanied by heart rates averaging 20 per minute and respiration rates of 2 per minute. These rates are definitely higher than those of the normally hibernating marmot at the same cell temperature, and are more nearly those found with animals under nembutal having a rectal temperature of 10° C.

No measurements were made of the heat production of an animal anesthetized by carbon dioxide, but as the heart and respiration rates and rectal temperatures were depressed, it is valid to assume that the heat production was also lowered.

Conclusions. From seven experiments on six marmots we conclude that, when subjected to cold and to a mixture of from 20 to 45 per cent carbon dioxide plus either air or oxygen, the marmot becomes narcotized within an hour, its heart and respiration rates are lowered and its rectal temperature falls. At least 20 per cent carbon dioxide is required to maintain narcosis. This narcosis is not a result of an oxygen deficiency. Although the rectal temperature is not lowered by the narcosis, as such, it does follow the trend of the heart and respiration rates, from which it is concluded that the fall in body temperature is a result of and not the cause of the lowering of the metabolic processes. When the carbon dioxide is entirely discontinued, the animal regains consciousness and its body functions return to normal. When the narcosis was continued, the animals died within 80 hours. The body functions of a marmot are more intense under carbon-dioxide anesthesia than in deepest normal hibernation, but simulate well the conditions of this animal under nembutal.

THE CAUSES AND THEORIES OF HIBERNATION

The voluminous literature on hibernation has been commented on by many writers. In every research, no matter to what extent it was carried out or what phase of physiology or anatomy was studied, it would appear that every investigator has felt a moral obligation to use his data to formulate some theory or to offer some form of explanation as to the causes of hibernation. Often this was based upon extremely crude and wholly unconfirmed experimental data. The theories with regard to hibernation are numerous and are perhaps best brought together in the excellent article by Rasmussen¹ published in 1916 with his comments on the validity of the several theories, based on his judgment primarily as an anatomist. A comprehensive article, published in 1931, by Johnson² discusses in detail, with numerous citations, the literature on the marmot with a long series of discussions as to the conditions and possible causes of hibernation. This includes not only the work of earlier writers but much of Johnson's own experimenting, chiefly temperature regulation carried out with extraordinary precision.

Polimanti,³ who wrote one of the two great volumes on hibernation (the other the work of Dubois)⁴ to which because of language difficulties we are unable to do justice, gave an address before the Vienna Biological Society on March 2, 1936,⁵ presenting in German much of the material in his valuable monograph. In this address, of the several topics treated, Polimanti laid emphasis upon hibernation. Thus we have a 1916, a 1931, and finally a 1936 presentation of theories. It is curious that the two last mentioned compilations consider, with sufficient degree of seriousness to report in not a little detail, the alleged hibernation or suspended animation or deep winter-sleep of humans, with particular citation of the reported winter-sleep habits of the Russian peasants and also of the Indian fakirs. It is true that Johnson gives a rather reserved presentation of the subject, whereas Polimanti lays considerable stress upon it, with obvious conviction but without proof of the alleged facts. Since most of the earlier studies of hibernation had to deal with anatomy, changes in body weight, and certain temperature measurements, the discussion has for the most part been limited to this type of phenomena. In our own work emphasis from the start was laid upon the heat production on the general theory that the heat production is the best measure of vital activity and that in the hibernating animal we are dealing with tremendous variations in the vital activity which could be best and most accurately measured only in terms of heat production. Frankly, the heat production could not be measured by direct calorimetry for a num-

¹ Rasmussen, A. T., Am. Naturalist, 1916, 1, p. 609.

² Johnson, G. E., Quart. Review Biol., 1931, 6, p. 439.

³ Polimanti, O., *Il Letargo*, Roma, 1912.

⁴ Dubois, R., *Physiologie comparée de la marmotte*, Paris, 1896.

⁵ Polimanti, O., Wiener klinische Wochenschrift, 1936, Jahrg. 49, No. 30, p. 925.

ber of technical reasons. Buttressed by recent observations on the goose,^{6a} when the respiratory quotient was far above the normal range, exceeding at times 1.4, in which the extreme accuracy of indirect calorimetry was verified, we feel the heat production here reported has been accurately established.

Our main duty was to secure the facts, under the best conditions possible, and to record them accurately, realizing that ultimately explanations and theories will have a better basis than if the time is devoted to speculation with a minimum of attested fact. Emphasis has already been laid upon certain, at least, of the salient points contributing toward the mental attitude of research workers in dealing with the laws underlying hibernation. For example, such outstanding respiratory quotients during hibernation as those approaching 0.30 have previously been explained theoretically by the transformation of fat into carbohydrate. The new experimental evidence here presented disproves such quotients conclusively. We are fully aware, as a result of our experimenting, that the technical difficulties of measuring the gaseous metabolism of a large marmot, and incidentally this is practically the largest hibernating mammal available to man, are such as to explain very easily most of the experimental difficulties experienced by many of the earlier workers. Unfortunately most writers have been rather loath to give up the false impressions arising from these difficulties. It remains a fact that many observations similar to ours have been reported by other investigators in the vast amount of experimenting that has previously been carried out (most of which does not include measurements of the respiratory exchange), and we would in no wise wish to detract from or lessen the due credit that should be given to them. Had it not been for the wide diversity of findings and interpretation of results, we could have accepted as established certain of the physiological phenomena such as the respiration rate, the changes in body weight, and the heart rate, and a great deal of our work would have been unnecessary. So far as the studies of respiratory exchange and the calculation of heat production therefrom are concerned, the situation is somewhat different, since we find the earlier work, save with rare exceptions, is of little positive value. This does not mean that certain of our findings dealing with respiratory exchange have not already been stated by earlier writers, although critical analysis will show their deductions were based upon wholly inadequate data. The question arose whether or not this more refined index of vital activity, namely, heat production, would contribute new evidence as to the causes of hibernation or illuminate the theories regarding it. Consequently we will pass over rather rapidly a discussion of those theories and causes in which the simpler, physical measurements are used as criteria. However, before taking up the theories of hibernation we are presenting here a digest of our main findings, again emphasizing our great indebtedness to earlier writers and regretting this cannot in many cases be manifested by numerous literature citations in a book already too voluminous.

^{6a} Benedict, F. G., and R. C. Lee, Carnegie Inst. Wash. Pub. No. 489, 1937, p. 215.

DIGEST OF MAIN FINDINGS

The non-hibernating marmot in the post-absorptive state has a minimum heart rate of 80 beats per minute, and an average minimum respiration rate of from 25 to 30 per minute. The rectal temperature is labile, ranging from 34° to 39°, but has a general trend to average 36° at 16° C. environmental temperature, 37° at 28° C., and 38.5° when the environmental temperature is above 30° C. The insensible loss at an environmental temperature of about 17° C. averages 7.2 gm. per kilogram per 24 hours and 15.7 gm. per kilogram at 31° C. The nitrogen excretion is 0.5 gm. per kilogram per 24 hours when the marmot is normally feeding. Forty-eight hours must elapse after the animal eats to insure respiratory quotients of 0.71. The zone of thermic neutrality is confined to almost a single point at 28° C. The minimum basal metabolism is 410 calories per $10w^{\frac{1}{2}}$ per 24 hours, there being no difference between day and night and no close correlation with the rectal temperature. The heat lost by vaporization of water at the critical temperature is 20 per cent of the total. At 16° C. the minimum heat production averages 899 calories per $10w^{\frac{1}{2}}$. The major adjustment of the heat production to the environmental temperature is very rapid.

Prolonged fasting of marmots showed survival time averaging 110 days, with several animals continuing to 165 days. The average weight loss at death was 56.5 per cent of the initial body weight and the maximum 72 per cent. The absolute minimum respiration rate of 10 per minute occasionally observed in the basal non-hibernating state is lowered to 4 in prolonged fasting. Their heat production during the fast is most uniform on the $10w^{\frac{1}{2}}$ basis, tending to drop only slightly. When the marmot is kept at the critical temperature, the rectal temperature is maintained fairly normal even near the end of fasting.

The marmot entering hibernation is characterized initially by a series of downward steps in physiological functions, as shown by the rectal temperature, usually returning to its non-hibernating state several times. The heat production is partially lowered, as is the rate of respiration. The animal frequently appears drowsy in this stage. Fat is the source of energy during this time, but the respiratory quotient is apparently below the level characteristic of fat combustion due to retention of carbon dioxide by the cold tissues. The most practical index of the phase of vital activity of the animal, although not strictly accurate, is the respiration rate.

In deep hibernation the minimum heart rate is 4 to 5 beats per minute. The minimum respiration rate is 0.2 per minute, the average being not more than 1 per minute. The rectal temperature may be as low as 3° C. The absolute minimum heat production is 17 calories per $10w^{\frac{1}{2}}$, although 27 calories per $10w^{\frac{1}{2}}$ is the average minimum attained by many animals. The low values of the heat production are not accompanied by the lowest rectal temperatures but frequently are observed at rectal temperatures of 8° to 12° C. Any gains in weight are primarily due to adsorption of water on the fur. The true combustion respiratory quotient is one of fat (0.70). The

excretion of nitrogen is very low, at times being 0.007 gm. per kilogram per 24 hours. The partition of the nitrogen excretion is not altered during hibernation. The heart rate is the function most closely correlated with the heat production.

Waking from hibernation may be the result of warming, handling, faradic shock, or internal factors. The process is a rapid one, the rectal temperature rising from the hibernation level to approximately 37° frequently in 2 hours. The heart rate ascends to a value over 200 per minute, a value above the maximum occasionally observed when the marmot is normally awake. The respiration rate increases above the normal basal rate but frequently falls quickly to a sub-basal level. The heat production rises extremely rapidly with almost explosive violence, usually reaches 2000 calories per 10w³ and may reach 4000 calories. This "peak metabolism" is usually maintained for half an hour or more. Fat is the chief source of this energy.

WINTER SLEEP OF THE BEAR

Our experience with the marmot in hibernation would base a definition of *deep hibernation* on a lowering of the body temperature to within a few degrees of the environmental temperature, accompanied by a profound decrease in the heat production. This condition would obtain only when the body temperature is 12° or below. Deep torpor representing these defined conditions is usually found only with small animals ranging from the ground squirrel to the 2- to 5-kg. marmot. It therefore becomes of special interest to examine more closely the commonly expressed belief that the bear hibernates. The evidence is very contradictory, although the generally prevailing opinion is that the bear does hibernate. Through the kindness of Mr. Shaler E. Aldous our attention was called to his recent observations on black bears^{6b} and to a report of Mr. Marius A. Morse of the bears in winter quarters. We are indebted and deeply grateful to them for placing their valuable protocols in our hands with permission to use and publish the data.

M. A. Morse, Junior Biologist of the Minnesota Civilian Conservation Corps and O. Kuehne, field assistant, report two groups of black bears in a condition which they describe as "hibernation." On January 3, 1936, with the air temperature at -5° C. in Cloquet Valley State Forest in northern Minnesota, 32 miles north of Duluth, an adult bear and three yearling cubs, each about 2½ ft. in length (76 cm.), were discovered in an open black spruce swamp protected by trees. During the first observation of 20 to 30 minutes, the adult bear opened its eyes, surveyed its surroundings unperturbedly and, after moving twice, resumed its sleep. Although snow was on the ground to a depth of 13 inches, there was none on the bears and no indication of their having moved about to shake it off. During the night a light snow fell and some remained on the animals when observed the follow-

^{6b} Aldous, S. E., Journ. Mammal., 1937, 18, p. 466.

ing day. The ordinary conversation of three persons did not arouse the animals, but shouting and whistling awakened the cubs, causing them to raise their heads, quickly followed by "cuddling down" to the big bear. Several minutes later the cubs ambled off one by one through the light snow. When they had gone about ten yards, the adult bear awoke, slowly surveyed the situation, groggily moved to the cubs and took the lead. The four bears then went deeper into the swamp and out of sight. Three days later within 200 yards of the previous "bed" the family was found. The adult bear was quite awake and unfriendly.

The group was found in sound sleep on January 15, 1936, when the air temperature was -26° C. There was no evidence on the bears of an eight-inch snowfall two days previous, despite the cold weather. Whether it had melted or was shaken off was not apparent. Two weeks later the bears were still asleep, with little change since the last observation.

On February 10, the adult bear, although sleeping when first observed, awoke and stirred slightly. This was again noted on February 13 at a temperature of -21° C., and at this time the large bear had a respiration rate of 4.5 per minute and two of the cubs had rates of 4 and 5 per minute. Though snow had been falling all morning, there was but little on their bodies.

Expeditions on February 18, March 8 and March 19 found the adult awake and some of the cubs exhibiting signs of movement, the temperature being slightly above freezing on the last two days. The animals were visited on March 26 and April 9, some being awake on each day. By April 15, the bears had left their bed.

Another bear was found in a den on February 3, 1936, with evidence of having recently been out of the den. On a visit on February 22 the den was found sealed by snow. On opening a small hole the observers could detect no reaction to sound or to snow falling on its nose on the part of the bear.

The den on April 20 was partially filled with water and contained no bear, but a short distance off three small cubs each about 10 inches (25 cm.) long were found. The presence of cubs was not discovered on February 3 or 22, although it is possible they were present.

Under the direction of Shaler E. Aldous, Assistant Biologist of the Lake States Forest Experiment Station in Minnesota, Mr. Ivey Hartzberg made observations on a black bear found on January 29, 1937. This bear, reposing in a den under the arching roots of a dead white pine, was discovered because of the sound made by bear cubs, which were the size of a red squirrel. At this time the only bear cub that was visible was blind, and the claws were covered with a protective sheath. The mother bear was described as being in "hibernation" but other notes state, "apparently sleeping or in a lethargic condition, ready to move her legs or adjust her position at the least sound of discomfort from the cubs." The notes also attribute some of the crying of the cubs to losing the teat when nursing. In March the number of the cubs was definitely determined as three and by the 26th of the month the "animals had grown to the size of a medium-sized woodchuck."

The first evidence of the mother bear's leaving the den was noted on March 28th when tracks indicated she had traveled 12 feet to defecate. The animal ate fresh spruce needles on her way back to the den. There was no sign of the cubs leaving the den during March. April 5 revealed the whole group asleep. Numerous tracks indicated that departures from the den were frequent during the first part of April. On April 8th, apparently due to conditions in the den being too moist, the animals moved off 500 feet and made a new bed, which was used until the disappearance of snow left dry places in the vicinity.

These reports would emphasize the following facts about the bear in so-called "hibernation": (1) The bear goes into a sleep under conditions different from those of the marmot. It does not dig deeply into the ground to make a burrow down below the frost line, as its dens are frequently made near the surface of the ground merely under roots and branches covered with snow. Thus the bear more definitely lives at the temperature of the air rather than at that farther under the ground. (2) Its torpor is not so deep that it cannot quickly awaken and move about; it is semi-consciously aware of its surroundings. (3) The temperature of the bear's skin, even when the air is much below freezing, is warm enough to melt snow. (4) The bear has certain physiological functions going on during the so-called hibernation period to enable it to give birth to and to suckle young. In other words, the mammary glands are sufficiently active to produce milk, and the fact that snow falling on the body always melts gives evidence of a warm body temperature. Therefore, we conclude that bears, even when somnolent and unirritable, nevertheless cannot be in true hibernation, because they have not a low body temperature and a low heat production.

The question of whether or no the bear is a truly hibernating animal is far deeper than the desire of knowing if one other species of animal hibernates. It has been the custom of many writers, we think wholly unjustifiably, to lay undue stress upon the alleged suspended animation of the Russian peasant and particularly the Indian fakirs. If the supposed hibernation of the bear had been actually demonstrated with this animal, even larger than man, then there would be less reason to question the probabilities of man's hibernating. The facts are that no animal appreciably larger than the hedgehog or marmot has been definitely proved to be in hibernation.

FACTORS CONSIDERED

The present-day situation with regard to the explanation of hibernation was admirably expressed two decades ago by Rasmussen⁷ as follows: "While we congratulate the last few generations upon the rapid growth that has been made in scientific knowledge, there yet remain phenomena that are almost as unintelligible today as they were a hundred years ago—the most earnest and often tedious experimentation and observations of several generations having shed but little light on the factors and mechanisms

⁷ Rasmussen, A. T., Am. Naturalist, 1916, 1, p. 609.

involved." To one who has been engaged in research upon this extremely puzzling problem, this phase remains as true today as it was twenty years ago.

Based upon the repeated emphasis of Polimanti,⁸ it is evident that a great mistake has been made in not recognizing the necessity of complete silence in the attempts to get our animals to hibernate. The sensitivity to sound of marmots in semi-hibernation and perhaps deep hibernation has been noted not infrequently in these observations. A common experience was to enter the garage, observe the animals in some stage of hibernation and, while working in the room, notice that some animals had become aroused. At times this was definitely associated with some noise incidental to the observations. Hitherto the significance of these noises in relation to hibernation has not been recognized. Although noise appears to hinder the entering into hibernation, animals in deep hibernation under observation did not awaken when sounds were intentionally made.

One important factor is that marmots naturally hibernate in a burrow and the burrow implies a quiet environment. The emphasis laid by Polimanti upon the necessity for quiet is well placed. It is a factor too long overlooked by most workers. Although our garage was isolated from the main building, it was far from sound-proof. There was considerable traffic in the street, trucks were passing and delivery wagons were in the neighborhood. The doors were open three or more times a day, and these animals were far from being in a quiet place. This failure to evaluate correctly the stimulus of noise is a defect in this research, and we are inclined to agree with Polimanti that complete silence may be, and probably is, a predisposing cause of hibernation, which has hitherto been overlooked and certainly should be a factor emphasized in future studies where attempts are made to hasten or induce hibernation.

The major causes inducing hibernation, recognized as practically essential by all writers, are *seriatim* as follows:

Cold. It is agreed by all that the animals must be placed in a cold environment. The optimum temperature is commonly stated to be from 10° to 11° C., almost exactly that found in our own researches. Although frequently temperatures as low as 0° and in a few cases above 11° were prevailing when some marmots hibernated, in general hibernation was found to take place more nearly between 5° and 10° C. than at a few degrees above or below this range. On the other hand, many animals were kept for weeks, if not months, at temperatures of 10° or slightly below without hibernating, which emphasizes the fact noted by many writers that cold is not certain to produce hibernation and that some other factor or factors enter into the initiation of this phenomenon. Some writers have been inclined to assert with considerable precision that 10° is the optimum temperature to induce hibernation and that a temperature a few degrees below this is distinctly disadvantageous. Our results will hardly justify such a sharp division of

⁸ Polimanti, O., Wiener klinische Wochenschrift, 1936, Jahrg. 49, No. 30, p. 925.

temperature ranges. The situation is complicated by the fact that the marmot only slowly and apparently insensibly loses its chemical heat regulation. Only when this heat regulation is lowest does it appear possible for the marmot to enter deepest hibernation. The lowest heat production is a measure of the depth of hibernation.

The conclusion can be drawn that cold *per se* is, in general, a contributory factor, but not of itself sufficient to cause hibernation.

Absence of food, or hunger. The second most generally accepted condition is that the animal should be without food. This is almost invariably mentioned as a cause, and yet not infrequently the opposite point of view is held. It is a well-known fact that animals rarely hibernate in zoological parks, and the normal explanation is that the animals always have plenty of food before them. This, therefore, would suggest that when food is available there would be no hibernation. A close study, however, of the natural habits of the marmot shows that the marmot frequently takes edible material, hay, into his burrow for bedding. In fact, marmots have been known to bring hay to the mouth of their den to be eaten on awaking, thus anticipating the need for food. Usually in the laboratory the evidence is again contradictory. Many writers emphasize that the animals will not hibernate with food before them, but others state that they will. In our efforts to secure as large a number of animals entering hibernation as possible, and based upon the experience of earlier writers, we obviously sought every available facilitating factor and hence, in the effort to induce hibernation, food usually was removed.

However, we have evidence to show that even when food was present some animals did hibernate, so that our conclusion would be that the absence of food is probably on the whole contributory, but not absolutely essential. This does not consider whether or no an animal *partaking* of food would hibernate. We have no evidence that the animals partook of food in semi-hibernation. In some marmot colonies animals have been noted to wake frequently from deep hibernation, defecate, urinate, and often pick up small amounts of food as they returned to their burrows. From this one could infer that the presence of a small amount of food in the upper part of the intestinal tract did not prevent their returning to hibernation. However, here again there are two distinct points at issue; one, when the animal first enters hibernation, and the second, when it re-enters after hibernating shortly before. It is conceivable that its food might keep it from going into hibernation for the first time, but once it has hibernated, the urge to go back into hibernation might be so strong that it would offset any stimulus of food. Of our population of twenty-five animals in the fall of 1936, two marmots entered hibernation with food available, but this was not the general case, for the other marmots did not hibernate under the same condition. When food was removed, all the marmots in the cold garage ultimately hibernated. Therefore, absence of available food, although again a facilitating factor, is not a requirement for obtaining hibernation.

Darkness. Knowing that for most hibernating animals the winter quarters are deep in the ground, away from light, one would suppose that a natural facilitating factor would be complete darkness. In some laboratories artificial burrows have been prepared, enabling the animals to simulate the normal condition. In our own experience the animals were in a garage with black cloth over the windows. Except for the time when the operators were in the room cleaning out the cages, weighing and measuring, under which conditions the garage was lighted by electric lights, usually from 30 minutes to two hours daily, our animals were for the most part in Stygian darkness. They were subjected to this light stimulus from time to time, with no particular precautions taken to keep the light away from the animals during our observations. As Johnson points out, although it has been the custom for most investigators to keep their animals in the dark, light has not prevented hibernation, and Johnson in a series of experiments on twelve animals showed that there was no effect of light. The tendency for the hibernating animal to roll up, somewhat like a ball, with the nose near the anus under the body, would tend to keep light away from the eyes, and the eyes are always closed in normal hibernation. Although absence of light *per se* may not, therefore, be considered as a contributory cause, no investigator would place an animal in direct sunlight, not simply on account of the fact that the direct sunlight would warm the animal, but on account of the great contrast between the light, particularly of the sun, in the day-time and the darkness at night.

Confined air. Based upon earlier experiments, chiefly those of Dubois, in which the auto-narcosis by carbon dioxide was considered to be one of the predisposing causes, the theory has developed that the accumulation of carbon dioxide in the burrow is an important factor in hibernation. In our own experiments nothing of the sort could possibly enter into the picture. The animals were always in open cages with no possibility of an increment of carbon dioxide of any considerable amount. To test the possibility of a high percentage of carbon dioxide in the concrete garage, with well-fitting doors, which housed at this time twenty-five non-hibernating animals, the air was analyzed after the garage had been closed over night and the percentage of carbon dioxide in the air was but 0.11 per cent, this being wholly without any physiological significance. It has also frequently been observed that many hibernating animals hibernate in open nests. Hence accumulation of carbon dioxide could hardly be considered, in the light of present-day knowledge, a contributory cause. This is quite aside from the fact that the carbon-dioxide content of the blood may alter considerably. As has been shown by Polimanti, Rasmussen, and others, the pH of the blood rises during hibernation and the urine becomes acid as the animal becomes carnivorous, subsisting upon its own flesh during the long period of fasting associated with hibernation. From experiments with marmots in various carbon-dioxide-containing atmospheres, it is concluded that carbon dioxide plus cold can produce a condition of torpor characterized by a lowering of the body

temperature and decreases in heart and respiration rates, but the condition cannot be considered true hibernation for when the carbon dioxide is discontinued the animal regains consciousness. This carbon-dioxide narcotization does not hasten normal hibernation, and all of our data would disprove confined air or a carbon-dioxide increment as even an aid to the inducement of the true hibernation.

Absence of external stimuli. Daily experience with these animals emphasized their extreme susceptibility to external stimuli. Many attempted experiments had to be discontinued because the handling of the animal, to attach electrodes or insert a thermometer, almost invariably stimulated the animal to wake, and thus defeated the purpose of the experiment. Not only these necessarily pronounced external stimuli, but the mere transfer from the cage in which they were confined to the balance, for example, would frequently result in the animal's being stirred up or stimulated. On one occasion, at a social affair in the laboratory, a marmot was brought from the cold garage, apparently in deepest torpor, curled up in a desiccator with the cover removed. It was rapidly carried around the room where the guests could see the animal, and one venturesome lady touched the animal, which immediately responded by a body movement and a chattering noise. We also noted that sound frequently disturbed the animals not in deep hibernation. The ideal experimental situation for most of our work was to have the animal in deep hibernation, with a thermo-junction in the rectum and electrodes attached to the paws for heart-rate determinations. This situation could be obtained with some animals, but there was a very great difference in individuals, as most animals were awakened by making these attachments, and did not immediately re-enter hibernation. Not infrequently animals destroyed thermo-junctions or removed electrodes when partially aroused from hibernation by the attachment of this equipment. Therefore, the minimum amount of handling would be advantageous toward continued deep hibernation. This of itself militates against as complete a series of physiological observations as desired, as it rules out rectal temperature measurements and the attachment of heart electrodes, and explains in part why the data, in spite of our large population, are rather limited on some of these points. Clearly then, the absence of external stimuli, either by touch or by sound, is a contributory cause to induce or maintain hibernation.

Sleep. Although it is impossible in this monograph to discuss in detail or even to cite the extensive literature on the question of sleep as such, it should at least be emphasized that the close relationship between winter sleep or hibernation and the normal sleep of humans has been rather grossly exaggerated. A comatose condition is by no means synonymous with sleep, certainly not with human sleep. An excellent illustration of this exaggeration is the common assumption that hypnotic sleep is another form of human sleep. A study of the metabolism of human subjects in hypnotic sleep⁹ shows that

⁹ Whitehorn, J. C., Lundholm, H., Fox, E. L., and F. G. Benedict, New Eng. Journ. Med., 1932, 206, p. 777.

this is by no means to be compared directly with normal sleep. In normal sleep with humans the metabolism is lowered about 10 per cent, but in so-called hypnotic sleep no such lowering takes place. This is not the place to discuss the thresholds to sound or other stimuli existing in relation to normal sleep or hypnotic sleep, but certainly neither hypnotic sleep nor hibernation can be directly compared to the sleep of humans. The data here presented should immediately tend to eliminate the common practice of making these wholly unjustifiable comparisons.

Season. A study of the life cycle of the marmot shows that these animals begin in the summer to lay up stores of fat for winter, derived from food predominantly carbohydrate, and are usually ready for hibernation in a very well-fattened condition. Hibernation in the New England states, for example, takes place in the early autumn, September or October. The animals then remain in hibernation with occasional appearances, to which legend has attached a most interesting story in connection with the appearance of the marmot above ground on February 2—locally called Ground Hog Day.¹⁰ Usually it is in hibernation for five to six months, existing on body materials. As vegetation appears with the coming of warm weather in the spring, the marmot comes out of hibernation. As we have seen, in the awakening stage there is an enormous heat production which, when the marmot is fully awake, decreases to the normal, non-hibernating level. Thus throughout the winter there are drafts on body stores. It is an interesting fact that the rutting season takes place immediately after the marmot comes out of hibernation.¹¹ The females are impregnated and the young born shortly thereafter, as shown by one case where young were born in the laboratory (see page 8). The mating season of these marmots immediately following a prolonged draft upon body stores, which would be interpreted as lowering the physical state, is at variance with that of most other animals. Perhaps a most striking contrast would be the Alaskan seals, the males of which species return to the breeding grounds well fattened and in superb condition for the combats of the mating season.

Consequently the normal time for hibernation for the marmot would be in the late fall. From the rather scattered observations on the marmots under the special conditions maintained in the laboratory, the impression was that some of the animals were quite inclined to go into hibernation in the middle of December. Among our larger population in 1936 and 1937 there was still a tendency for many of the animals to enter hibernation about the middle of December, perhaps a month or six weeks later than they would outdoors. This has already been explained by the fact that in the laboratory the temperature was warmer, food was available later in the season, and there were some stimuli, so there is not necessarily a fixed period

¹⁰ The myth claims that on this day the ground hog (marmot) comes out of its burrow for the first time since hibernation. If the animal sees its shadow, this is a sign that winter will continue for six weeks, so it returns to hibernation again.

¹¹ Rasmussen, A. T., *Endocrinology*, 1921, 5, p. 33.

when these animals enter hibernation, uninfluenced by other factors. In our group of animals, however, a goodly number did not enter hibernation until much later in the year, sometimes as late as February. The most extreme case of this shift in the season was with one animal that did not hibernate in captivity during the winter (it had been dug out) brought to the laboratory on December 11, and was fed all winter, gaining a large amount in weight). This marmot was given food, kept in a warm room, and therefore had no inclination to hibernate. Food was removed on May 15 and the marmot was placed in a refrigerator at a temperature of 8° to 10° C., remaining there (awake) for about four or five weeks in complete darkness and quiet, with but the intermittent vibration of the electric refrigerator. About June 20 the animal began to hibernate, and was recorded as being in deep hibernation about July 1. This hibernation was maintained with only occasional wakings, except when the animal was stimulated by handling, until well into October. It was used for some heart-rate studies, but died shortly thereafter, being emaciated.

This, therefore, demonstrates that we are not dealing with a purely seasonal effect, for by these more or less artificial conditions the hibernating season has been distributed throughout a good part of the year. However, emphasis should be laid upon the fact that marmots have a tendency to be in hibernation, even in captivity, before the middle of December.

Other factors. Since our observations involved no operative procedures and no histological studies, we have no contribution to anatomical findings. Many writers maintain there are no gross anatomical changes due to hibernation. Cushing and Goetsch,¹² however, stress that the hypophysis of the marmot undergoes a decrease in hibernation. From the chemical standpoint our experiments show that one commonly supposed change in metabolism due to hibernation, that is, a marked change in the character of the respiratory exchange involving (a) a lowered respiratory quotient, (b) a retention of oxygen, and (c) a perturbed oxidation of body material, does not exist. Furthermore, from Carpenter's study of the partition of nitrogen it is clear that the protein metabolism undergoes no qualitative alteration in hibernation. These two purely chemical findings strongly support the belief that there are no profound anatomical or functional changes associated with hibernation, as such, and all theories involving such an assumption are, therefore, invalidated.

Of the innumerable other supposed or alleged contributory causes, many of them deal with anatomical, nervous, or endocrine factors that are not adequately covered by our own study, and hence discussion is not justifiable.

Rasmussen, in commenting upon the diversity of opinion and the relative merits of the various theories, states that "all of them are based upon insufficient data. To say which of the various conditions associated or occurring simultaneously with winter sleep are concerned with the production of

¹² Cushing, H., and E. Goetsch, Journ. Expt. Med., 1915, 22, p. 25.

the lethargy or which are the results of this or some other conditions is extremely difficult."

The whole picture of the hibernating marmot is one of a decrease in vital functions, and the question may be asked, what can slow the vital functions? When one substitutes for vital functions metabolic activity or heat production, then the problem results in, what are the demands or causes for metabolism? Why is the metabolism lowered?

Our research clearly demonstrates that the normal, non-hibernating marmot, with a cell temperature of 37° C., has a basal metabolism, on any basis of calculation, far lower than any other warm-blooded animal of approximately the same size. For instance, a 3-kg. marmot would produce two-thirds of the total calories per day produced by a rabbit or one half that by a goose of similar weight. This animal has an initial non-hibernating metabolic level far below that of practically all other warm-blooded animals, and we have then to revert to our bridge simile—an animal that is in the non-hibernating condition starts at an extremely low level for crossing the chasm. Consequently one might assume that the non-hibernating marmot, due to its anatomical structure or its circulation needs, is especially designed for a low metabolic level and that any factors inducing a still lower level would be imposed upon an animal attuned to adapt itself to such a situation. This is not true for most homiotherms.

Under conditions where the marmot does not have to produce heat to combat the cold, *i.e.*, at 28° C., its metabolism is at a very low level. When the situation demands chemical heat regulation, the marmot shows an instantaneous response to the lower air temperature prior to entering into hibernation, a response that is very great when one takes into consideration the size of the animal, the heavy fur covering, and the heavy layer of fat. The importance of this highly activated chemical regulation in preventing the unconscious animal from freezing to death, when the environmental temperature descends to too low a level, has already been emphasized. This is a peculiar feature of the marmot, and the intensity and effectiveness of its chemical regulation is approached by no other animal. It would seem as if the marmot attuned to a low metabolic level had, to compensate for this characteristic, a supersensitive chemical regulation to keep the body temperature up and prevent its falling to a dangerous point in extremely cold environments.

This adjustment of the body to a low heat production bears close resemblance to the well-known low heat-producing capacity of the cold-blooded animals, particularly the snake. It has been demonstrated that the snake, even when warmed to the cell temperature of the warm-blooded animal, cannot produce an equivalent amount of heat. Under this condition the heat production is usually only about one-eighth that of a warm-blooded animal of the same weight and same cell temperature. This has been explained on the ground that there is, in the first place, an extremely small blood supply in the snake. Certainly there is an extremely small blood

distribution. The white, pale-colored flesh of the snake has been pointed out as being practically without coloring matter from the blood, which suggests that very small amounts of blood are in circulation. In the marmot, anatomists' findings (supported by temperature measurements) have shown that here again the blood circulation is poor and differently subdivided. Two entirely different methods of attack have demonstrated this. First, the injection of coloring matter, such as indigotin used by Mareš,¹³ shows that the distribution of color is by no means perfect. Secondly, when the animals are waking from hibernation with a rapid temperature rise, measurements of the body temperature in the rectal passage (see page 185) at different depths between 25 and 150 mm. from the anus indicate that the heat production increases more rapidly in the central part than in the posterior part of the body. This condition is strikingly at variance with that of normal, warm-blooded animals, and represents a particular anatomical structure.

The difference in the blood distribution showing the central part of the body as warmer than the posterior part is in line with the observations on the regaining of consciousness as the animal is waking, for the animal has frequently been noted to have the eyes open while the hind legs were dragging or still incapable of supporting the body. This, therefore, points strongly to an unequal blood distribution in the body as a whole, and consequently an unequal slowing of the blood distribution as the animal is going into hibernation. This also suggests that in hibernation there is primarily a profound disturbance of blood distribution. These animals have a low metabolism and go to their lowest levels simply because the blood does not get to the cells, bringing oxygen and food material. Consequently, to repeat, we are dealing here with a lowered blood distribution, a deficiency in oxygen carried to the cells, and a deficiency in metabolites,¹⁴ these deficiencies resulting in a lowered metabolism. This particular point, however, emphasizes the idea which is by no means definitely established, that the heat production is determined by the supply of oxygen and the supply of metabolites and not that the heat production is determined by the needs of the cells as a result of muscular activity. The question may be asked, if the marmot is deficient in blood supply and blood distribution, how can it even momentarily whip up its metabolism 100 times in 40 to 50 minutes? This would point toward the metabolism being increased by reason of the needs of the animal to warm up its body and get ready for either combat or forage. These two theories are antagonistic, that is, the heat production is deter-

¹³ Mareš, F., Bohemian Arch. Med., Journ. Advancement Med. Sci., Prague, 1889, 2, p. 458.

¹⁴ Some will consider this in the light of the metabolism of plethora, as expressed by Voit. We have a somewhat different meaning here, in that we are concerned not so much with the actual concentration of the metabolites in the blood as we are with the actual amount of blood getting into the tissues. This is not to be confused with the excess metabolites after a heavy feeding, for example, but deals solely with blood circulation. The composition of the blood in deep hibernation is reasonably well known.

mined, on the one hand, by the supply of oxygen and the supply of metabolites, and on the other hand, the heat is produced by needs of the body to be warmed up. Based upon the extreme elaboration of the anaerobic nature of metabolic changes during muscular work, as outlined by Hill¹⁵ and Meyerhof,¹⁶ it is not inconceivable that there may be, for a short time during the rapid changes of warming, heat produced in excess of the oxygen supply and as a result an oxygen deficit. If this were the case, however, one would expect profound alterations in the respiratory quotient, which are not found. The common belief is that the energy for anaerobic heat is supplied by carbohydrate. That this theory cannot well apply to the hibernating marmot is demonstrated by the fact that the marmot, in the first place, undoubtedly has a very poor supply of glycogen. It is further worthy of note that if there is an anaerobic metabolism, our indirect measures of heat in these periods would naturally be too low and would not represent the true heat production, as they are based upon measurements of the oxygen consumption. All this, however, points strongly against the belief that the heat production is determined by the needs of the muscles, and we again stress the conviction that the heat production is determined by the oxygen and metabolites supplied to the cells.

One is always inclined to associate increased metabolism with muscular activity, with the thought that it is very difficult for an animal to increase its metabolism without muscular activity. With the awakening of the hibernating marmot, there is a tremendous production of heat unaccompanied by any visible muscular activity. This heat production may be maintained for some time at a level ten times that of the normal non-hibernating animal and this heat is produced without the apparent intervention of or use of muscular activity. The animals may not be perfectly immobile at this stage; but there are only relatively slow and slight changes in body position. The rise of the metabolism ten times above normal, or one hundred times above the hibernating level, if due to a mechanism involving muscular activity, would call for tremendous muscular movements. In all probability the marmot under normal conditions could not produce heat at the rate of 4000 calories per $10w^4$ except for a very short time, since it could not dispose of the resulting heat. In our experiments where we chased the marmot around the room, we found that the animal simply could not stand it. It would get into a corner and pant vigorously, unable to lose the heat. It is, therefore, clear that in the waking process or more properly the "burning-up" process the production of heat is unaffected by muscular activity and is a different order of heat production from that normally existing in the non-hibernating animal.

From this evidence it would appear that the heat production is determined by the blood taken to the cells, which carries thereto oxygen and metabolites. Teleological needs would call for a rapid combustion to bring the animal's

¹⁵ Hill, A. V., *Muscular activity*, Baltimore, 1926.

¹⁶ Meyerhof, O., *Die chemischen Vorgänge im Muskel*, Berlin, 1930.

body up to a functioning temperature level for fight, flight, or forage. In order to increase the temperature there must be something to burn. In order to burn this something, there must be a supply of oxygen, so from that standpoint the metabolism is determined by the needs and demands and calls on the cells of the body to warm it up. From the other standpoint, however, if one can conceive of a stimulus whipping up the circulation and forcing the oxygen and metabolites to the cells, that of itself would be the cause. A clear concept of the nature of such a stimulus would aid in an explanation of the phenomena.

The cell temperature of the cold-blooded animals is controlled by the environmental temperature. The cell temperature, in turn, regulates the metabolism, which increases when the cell temperature rises. It is possible to warm a few of the poikilotherms to a cell temperature of 37° C. The python at a cell temperature of 37° has a heat production of about 87 calories per $10w^{\frac{1}{2}}$. At this same cell temperature the tortoise, on the basis of weight less shell, would have a higher metabolism, approximately 100 calories per $10w^{\frac{1}{2}}$. The non-hibernating marmot is adjusted for a low heat production despite the fact that it is capable of vigorous intense action, comparable to the lightning-like rapier flash of the python, and its minimum level is lower than that of any other warm-blooded animal of approximately the same weight. There is then a series of steps in heat production for a group of animals all having the same cell temperature (37° C.). This series would start at the upper end with the rabbit, for example, having a heat production of approximately 620 calories, followed by the marmot with 410 calories, then a larger step to the tortoise with 100 calories, and the snake with 87 calories would be at the lowest level. This descending series is fairly well correlated with the blood distribution, for where there is the best blood distribution, there is the greatest heat production. The question then arises as to what is the cause and what is the effect? Is the blood distribution of the marmot low because its demands are low, or is it low because it has combustible materials supplied at only a slow rate? We believe, in the case of the snake, that its metabolism is low because it cannot make it high. It does not have a sufficient volume of blood or circulatory apparatus to maintain a high heat production. In other words, an adequate mechanism is lacking. This would point to the insufficient blood supply as the cause.

In view of the present knowledge, the most logical explanation of the cause of heat production and of the differences in heat production between warm-blooded and cold-blooded animals lies in the differences in the supply of oxygen and metabolites to the cells. This supply is regulated (1) by the nutritive state, *i.e.*, the metabolites and oxygen in the blood, and (2) by the circulation and distribution of the blood to the tissues.

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BENEDICT, FRANCIS G., AND ROBERT C. LEE. *Hibernation and marmot physiology.* (Carnegie Institution of Washington Publication No. 497, 1938.) x + 240 pages, 2 plates, 11 figures, and 58 tables.

A metabolic survey of the marmot or woodchuck (*Arctomys monax*) was made to contribute information regarding the physiology of the animal itself and for comparison with other warm-blooded animals that do not hibernate and with cold-blooded animals. As the marmot may be living on one day as a normal warm-blooded animal and on another day more nearly as a cold-blooded animal, it bridges the gap, so to speak, between these two great animal classes. Forty-eight marmots, of both sexes, ranging in weight from 1 to 5 kg., were studied in the non-hibernating and hibernating states, and in the transitional stages of entering and awakening from hibernation. The measurements made included those of body weight changes, insensible perspiration, heart and respiration rates, rectal temperature, respiratory exchange, and water-vapor output. The effects of different environmental temperatures upon the various physiological functions were also studied, as well as the body composition after prolonged fasting. Observations were made on several marmots that were under nembutal and subsequently exposed to a cold environment and upon others that were subjected to carbon-dioxide narcosis and cold. The metabolism of the marmot under nembutal was measured and compared with that of the normal marmot in deep hibernation. Analyses were made of the urines of several marmots while fasting and hibernating, including determinations of the total nitrogen and the percentages of nitrogen in the form of ammonia nitrogen, amino-acid nitrogen, urea nitrogen, and preformed creatinine.

The study of the respiratory quotients was materially aided by the measurements made on marmots under the influence of nembutal. Contrary to the commonly assumed changes in metabolism due to hibernation, there was not a marked change in the character of the respiratory exchange, the respiratory quotient was not lowered,

there was no evidence of a retention of oxygen, and there was no hint of a perturbed oxidation of body material. The protein metabolism underwent no qualitative alteration in hibernation. These two purely chemical findings support the belief that there are no profound anatomical or functional changes associated with hibernation, and all theories involving such an assumption are, therefore, invalidated. The marmot, when non-hibernating, has an extremely labile basal heat production and a labile rectal temperature, but when its cell temperature is 36.9°C., its basal heat production averages about 400 calories per $10w^{\frac{3}{4}}$ per 24 hours. This is much lower than that of any other warm-blooded animal of approximately the same size thus far studied but is appreciably higher than that of any cold-blooded animal of similar size at a cell temperature of 37°C. The hibernating marmot simulates the cold-blooded animal to some extent in that when exposed to low environmental temperatures it assumes a very low rectal temperature and a very low respiration rate, but its heat production per $10w^{\frac{3}{4}}$, even at the lowest level, is at least two or three times that of the cold-blooded animal of approximately equal size having the same low cell temperature. The monograph concludes with a digest of the main findings and a consideration of the possible causes of hibernation.

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BENEDICT, FRANCIS G., ET ROBERT C. LEE. *Hibernation and marmot physiology.* (*L'hibernation et la physiologie de la marmotte.*) (Carnegie Institution of Washington Publication No. 497, 1938.) x + 240 pages, 2 planches, 11 figures et 58 tableaux.

On fit une étude de la marmotte (*Arctomys monax*), afin d'obtenir des données portant sur la physiologie de l'animal même, ainsi que pour faire des comparaisons avec d'autres animaux à sang chaud qui n'hibernent pas et aussi avec des animaux à sang froid. Vu qu'un jour la marmotte peut vivre comme un animal à sang chaud tandis qu'un autre elle se rapproche plutôt d'un animal à sang froid, elle sert de trait d'union, pour ainsi dire, entre ces deux grandes classes animales. On étudia 48 marmottes des deux sexes, dont le poids variait d'un à 5 kg., aussi bien lorsqu'elles n'hibernaient pas que durant l'état d'hibernation, ainsi que dans les phases intermédiaires de l'entrée et de la sortie de l'hibernation. On mesura les changements dans le poids corporel, la perspiration imperceptible, les fréquences cardiaques et respiratoires, la température du rectum, l'échange respiratoire et le dégagement de vapeur d'eau. On étudia aussi l'effet produit par les diverses températures ambiantes sur les différentes fonctions physiologiques, de même que la composition du corps après un jeûne prolongé. On observa plusieurs marmottes se trouvant sous l'influence du nembutal et puis exposées au froid, ainsi que d'autres soumises d'abord à une narcose d'acide carbonique et ensuite au froid. On mesura le métabolisme de la marmotte sous l'effet du nembutal et le compara avec celui de la marmotte normale dans un état d'hibernation profonde. On analysa les urines de diverses marmottes jeûnant et hibernant tout en déterminant la quantité d'azote total, ainsi que le pourcentage d'azote sous forme de N de NH_3 , N d'amine-acide, N urée, et N créatinine préformée.

Les mesures prises des marmottes sous l'influence du nembutal furent très utiles pour l'étude des quotients respiratoires. Contrairement aux changements dans le métabolisme communément attribués à l'hibernation, aucun changement évident n'eut lieu par rapport au caractère de l'échange respiratoire, il n'y eut pas de diminution du quotient respiratoire, aucune indication d'une retention d'oxygène et aucune trace d'une oxydation dérangée dans la matière corporelle. Le métabolisme de la protéine ne subit aucun changement qualitatif durant l'hibernation. Ces deux données purement chimiques viennent à l'appui de l'opinion qu'il n'y a point de profonds changements anatomiques ou fonctionnels associés à l'hibernation, donc toutes les théories comportant une pareille supposition se trouvent par cela rendues nulles. La production de chaleur de même que la température rectale de la marmotte, lorsque celle-ci ne se trouve pas en état d'hibernation, sont extrêmement labiles, mais lorsque sa température corporelle est de 36.9°C., sa production basale de chaleur atteint une moyenne de 400 calories par $10w^{\frac{3}{4}}/p^2$ par 24 heures. Ce chiffre est de beaucoup plus bas que chez n'importe quel autre animal à sang chaud qu'on a étudié jusqu'à présent et étant à peu près de la même grandeur mais bien plus élevé que chez n'importe quel animal à sang froid d'à peu près la même grandeur et ayant une température du corps de 37°C. En quelque sorte la marmotte hivernante simule l'animal à sang froid en cela que lorsqu'elle est exposée à une température ambiante basse, sa température rectale ainsi que sa fréquence respiratoire deviennent extrêmement basses, tandis que sa production de chaleur par $10w^{\frac{3}{4}}/p^2$, même au niveau le plus bas, est au moins deux ou trois fois plus élevée que chez l'animal à sang froid d'à peu près la même grandeur et ayant la même température corporelle basse. La monographie se termine par une discussion des données principales et par un examen des causes probables de l'hibernation.

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BENEDICT, FRANCIS G., UND ROBERT C. LEE. *Hibernation and marmot physiology.* (*Winterschlaf und Physiologie des Marmeliers.*) (Carnegie Institution of Washington Veröffentlichung Nr. 497, 1938.) x + 240 Seiten, 2 Tafeln, 11 Abbildungen und 58 Tabellen.

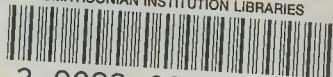
Ein Studium über den Stoffwechsel des Murmeltieres (*Arctomys monax*) wurde gemacht, um Auskunft über die Physiologie des Tieres selbst zu erhalten und auch zum Zweck eines Vergleiches mit anderen warmblütigen

Tieren, die keinen Winterschlaf abhalten, und mit kaltblütigen Tieren. Da das Murmeltier an einem Tage als ein normales warmblütiges Tier und an einem anderen Tag wiederum mehr als ein kaltblütiges Tier leben kann, überbrückt es so zu sagen die Kluft zwischen diesen zwei grossen Abteilungen der Tierwelt. Achtundvierzig Murmeltiere beider Geschlechter, deren Gewicht von 1 bis 5 kg. war, wurden ausserhalb sowohl wie während des Winterschlafes studiert, und auch im Übergangsstadium des Eingehens und des Herauskommens aus dem Winterschlaf. Die unternommenen Messungen begriffen Wechsel im Körpergewicht, Perspiratio insensibilis, Herz- und Respirationsfrequenzen, Rektumtemperatur, Gasaustausch und Wasserdampfabgabe. Man studierte ebenfalls die Wirkungen verschiedener Umgebungstemperaturen auf die verschiedenen physiologischen Funktionen, sowohl wie auch die Zusammensetzung des Körpers nach einer ausgedehnten Fastenperiode. Man beobachtete verschiedene Murmeltiere, die unter Nembutal waren und danach der Kälte ausgesetzt wurden, und wiederum andere, die erst einer Kohlensäure-Narkose unterworfen und darauf der Kälte ausgesetzt wurden. Der Stoffwechsel des Murmeltieres unter Nembutal wurde gemessen und dann mit demjenigen des normalen Murmeltieres im tiefen Winterschlaf verglichen. Chemische Analysen des Harns etlicher Murmeltiere während des Fastens und während des Winterschlafes ermöglichen Bestimmungen des Gesamtstickstoffs, sowohl als der Prozente von Stickstoff in der Form von Ammoniak-Stickstoff, Aminosäure-Stickstoff, Harnstoff-Stickstoff und vorhandenem Kreatinin.

Das Studium der Respirationsquotienten wurde erheblich durch Messungen, die an Murmeltieren unter dem Einfluss von Nembutal genommen wurden, gefördert. Im Gegensatz zu den üblich angenommenen Veränderungen im Stoffwechsel, die dem Winterschlaf zugeschrieben werden, fand hier keine wesentliche Veränderung im Charakter des respiratorischen Gasaustausches statt, eine Verminderung des Respirationsquotienten war nicht zu verzeichnen, kein Anzeichen eines Zurückhaltens von Sauerstoff war vorhanden und es war keine Spur einer gestörten Oxydation im Körpermaterial zu vermerken. Der Proteinstoffwechsel war keiner qualitativen Veränderung während des Winterschlafes unterworfen. Diese zwei rein chemischen Ergebnisse begründen die Annahme, dass keine beträchtlichen anatomischen oder funktionellen Veränderungen im Zusammenhang mit dem Winterschlaf vor sich gehen, so dass alle Theorien, die eine solche Annahme voraussetzen, ihre Gültigkeit verlieren. Das Murmeltier im vollwachen Zustand hat einen sehr ungestörten Grundumsatz, sowohl als auch eine ungestörte Rektumtemperatur, wenn aber seine Körpertemperatur 36.9° ist, dann ist seine Wärmeproduktion im Durchschnitt ungefähr 400 Kalorien pro 1 qm. pro 24 Stunden. Dieses ist bedeutend niedriger als bei irgend einem anderen warmblütigen Tier einer ungefähr gleichen Grösse, das man bis jetzt studiert hat, ist aber erheblich höher als bei irgend einem kaltblütigen Tier derselben Grösse mit einer Körpertemperatur von 37°C . Das Murmeltier, das im Winterschlaf begriffen ist, ähnelt dem kaltblütigen Tier einigermassen insofern, als dass wenn es einer niedrigen Umgebungstemperatur ausgesetzt ist, es eine sehr niedrige Rektumtemperatur sowohl als eine sehr niedrige Respirationsfrequenz aufweist, aber seine Wärmeproduktion pro 1 qm. sogar wenn am allerniedrigsten, ist immerhin noch zwei oder drei mal grösser als beim kaltblütigen Tier von ungefähr derselben Grösse und von einer gleich niedrigen Zelltemperatur. Die Abhandlung schliesst mit einer Besprechung der vorliegenden Ergebnisse und einer Erwägung der möglichen Ursachen des Winterschlafes.

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